

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



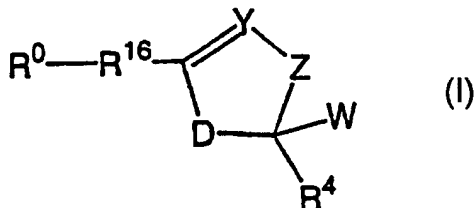
(43) International Publication Date
9 August 2001 (09.08.2001)

PCT

(10) International Publication Number
WO 01/56994 A1

- (51) International Patent Classification⁷: C07D 233/28, 271/06, 403/04, A61K 31/415, 31/41, 31/505, A61P 43/00
- (74) Agent: LINKKILA, Timothy, P.; Biogen, Inc., 14 Cambridge Center, Cambridge, MA 02142 (US).
- (21) International Application Number: PCT/US01/03347
- (22) International Filing Date: 1 February 2001 (01.02.2001)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
60/180,225 4 February 2000 (04.02.2000) US
- (71) Applicant (for all designated States except US): BIOGEN, INC. [US/US]; 14 Cambridge Center, Cambridge, MA 02142 (US).
- (81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.
- (84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): ZHENG, Zhongli [US/US]; 640 Marrett Road, Lexington, MA 02421 (US). CARTER, Mary, Beth [US/US]; 179 Wright Street, Arlington, MA 02474 (US). CUERVO, Julio, H. [CO/US]; 40 Columbia Road, Arlington, MA 02474 (US).
- Published:
— with international search report
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: INTEGRIN ANTAGONISTS



(57) Abstract: Novel integrin antagonists of Formula (I) are provided wherein a peptidic amide bond has been replaced, wherein all definitions are as in the claims.

WO 01/56994 A1

INTEGRIN ANTAGONISTS

FIELD OF THE INVENTION

The present invention relates to organic compounds, which are useful for blocking the activity of integrin molecules. This invention also relates to compositions
5 containing such compounds and methods of treatment using such compounds.

BACKGROUND OF THE INVENTION

Many physiological processes require that cells come into close contact with other cells and/or extracellular matrix. Such adhesion events may be required for cell activation, migration, proliferation and differentiation. Cell-cell and cell-matrix
10 interactions are mediated through several families of cell adhesion molecules including the integrins. The integrin very late antigen (VLA) superfamily is made up of structurally and functionally related glycoproteins consisting of (alpha and beta) heterodimeric, transmembrane receptor molecules found in various combinations on nearly every mammalian cell type. (for reviews see: E. C. Butcher, *Cell*, **67**, 1033
15 (1991); D. Cox et al., "The Pharmacology of the Integrins." Medicinal Research Rev. (1994) and V. W. Engleman et al., 'Cell Adhesion Integrins as Pharmaceutical Targets.' in Ann. Report in Medicinal Chemistry, Vol. 31, J. A. Bristol, Ed.; Acad. Press, NY, 1996, p. 191). Adhesion molecules of the VLA family presently include VLA-1, -2, -3, -4, -5, -6, -9, -10, -11, -v in which each of the molecules comprise a $\beta 1$
20 chain non-covalently bound to a α chain, ($\alpha 1$, $\alpha 2$, $\alpha 3$, $\alpha 4$, $\alpha 5$, $\alpha 6$, $\alpha 9$, $\alpha 11$, αv), respectively. The integrin $\alpha 4\beta 7$ is also intended to be included within the VLA family.

Such molecules play an essential role in both normal and pathophysiological processes in a wide variety of tissues. For instance, cellular adhesion and trafficking across the vascular interface plays an essential role in both physiological and
25 pathophysiological processes of acute brain injury. (Garcia et al 1994, *Am. J. Pathol.* 144:188; Becker et al, 1997 *PNAS* 94:10873). Further, leukocyte migration into glomeruli is a typical feature of human glomerulonephritis (GN) and leukocytes are key mediators of kidney damage. Therefore, the targeting of specific and relevant molecules in certain disease conditions without interfering with normal cellular
30 functions is essential for an effective and safe therapeutic agent that inhibits cell-cell and cell-matrix interactions.

VLA-1, -2, -4, -6 and $\alpha 4\beta 7$ neutralizing antibodies or blocking peptides that inhibit the interaction between these respective VLA moieties and their ligands are

known. Moreover, in the case of VLA-4 ($\alpha 4\beta 1$) and its ligand VCAM-1, some antibody antagonists have proven efficacious both prophylactically and therapeutically in several animal models of disease, including i) experimental allergic encephalomyelitis, a model of neuronal demyelination resembling multiple sclerosis
5 (for example, see T. Yednock et al., "Prevention of experimental autoimmune encephalomyelitis by antibodies against $\alpha 4\beta 1$ integrin." Nature, 356, 63 (1993) and E. Keszthelyi et al., "Evidence for a prolonged role of $\alpha 4\beta 1$ integrin throughout active experimental allergic encephalomyelitis." Neurology, 47, 1053 (1996)); ii) bronchial hyperresponsiveness in sheep and guinea pigs as models for the various
10 phases of asthma (for example, see W. M. Abraham et al., ' $\alpha 4\beta 1$ -Integrins mediate antigen-induced late bronchial responses and prolonged airway hyperresponsiveness in sheep." J. Clin. Invest., 98, 776 (1993) and A. A. Milne and P. P. Piper, 'Role of VLA-4 integrin in leukocyte recruitment and bronchial hyperresponsiveness in the guinea-pig." Eur. J. Pharmacol., 282, 243 (1995)); ix) tumor metastasis (for examples, see M.
15 Edward, "Integrins and other adhesion molecules involved in melanocytic tumor progression.", Curr. Opin. Oncol., 7, 185 (1995)).

Several peptidyl antagonists specific to VLA-4 have been described (D. Y. Jackson et al., "Potent $\alpha 4\beta 1$ peptide antagonists as potential anti-inflammatory agents', J. Med. Chem., 40, 3359 (1997); U.S. Patent 5,510,332, PCT Publications
20 W097/03094, W097/02289, W096/40781, W096/22966, W096/20216, W096/01644, W096/106108, and W095/15973). These peptide mimetic compounds of necessity contain the peptidic amide bond. See S.P. Adams and Roy R. Lobb, Annual Reports in Medicinal Chemistry, 34: Chapter 18, Academic Press, 1999. The peptidic amide bond is metabolically labile to hydrolysis and this limits the therapeutic utility of such
25 amide bond-containing molecules.

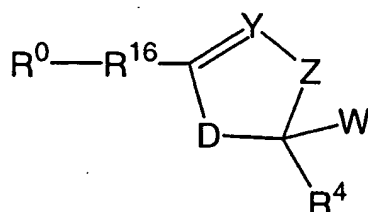
Notwithstanding the fact that peptidyl antagonists to integrins have been prepared, there remains a need for low molecular weight, specific inhibitors of integrins in which the amide bond is modified so that it is no longer susceptible to hydrolysis but contains the features important for integrin antagonist biological activity

30 SUMMARY OF THE INVENTION

We have developed antagonists of integrins with the advantageous property of lacking a peptidic amide bond. We have developed methods of using these antagonists that are useful in inhibiting integrins such as $\beta 1$ subunit

containing integrins as well as the integrin $\alpha 4 \beta 7$ and, in so doing, are useful in inhibiting cell adhesion processes including cell activation, migration, proliferation and differentiation. One aspect of the invention is a compound having the following structural Formula I:

5



wherein

10 D is selected from

- 1 $\text{NR}^j\text{-(CR}^k\text{R}^m)_m$;
- 2 $\text{S(O)}_n\text{-NR}^j$;
- 3 $\text{NR}^j\text{-O}$;
- 4 S-S ;
- 15 5 $\text{NR}^j\text{-NR}^j$
- 6 $(\text{CR}^k\text{R}^m)_m\text{-O}$;
- 7 $(\text{CR}^k\text{R}^m)_m\text{-NR}^j$
- 8 $\text{O-(CR}^k\text{R}^m)_m$
- 9 $(\text{CR}^k\text{R}^m)_m\text{-(CR}^k\text{R}^m)_n$
- 20 10 $\text{S(O)}_n\text{-(CR}^k\text{R}^m)_m$
- 11 $(\text{CR}^k\text{R}^m)_m\text{-S(O)}_n$
- 12 $\text{NR}^j\text{-S(O)}_n$
- 13 O-NR^j
- 14 NR^j
- 25 15 O ;
- 16 S(O)_n
- 17 C(O) ;
- 18 $\text{NR}^j\text{C(O)}$; or
- 19 C(O)NR^j

30

n is an integer from 0 to 2;

m is an integer from 1 to 2;

5

W is selected from

- 1 $-(CR^fR^g)_nC(O)OR^d$
- 2 $-(CR^fR^g)_n5\text{-tetrazolyl}$ or
- 3 $-(CR^fR^g)_nS(O)_2NHR^d$

10

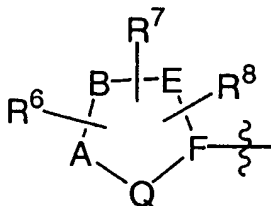
X is selected from $S(O)_2$, $S(O)_2NR^c$, $C(O)$, $C(O)O$, $C(O)NR^c$, CR^fR^g

Y is selected from N, CR^j

15 Z is selected from

- 1 $(CR^kR^m)_n$
- 2 NR^j
- 3 $S(O)_n$
- 4 O; or
- 20 5 $C(O)$

R^0 is selected from R^4 and



25

A and E are independently selected from $-C-$ and $-C-C-$, $-C=C-$;

F is selected from N, CR^2

Q is selected from $-CR^i-$, $C(O)$, O, $S(O)_n$, NH, NXR^l

30 B is selected from the group consisting of

- 1 a bond,
2 -C-,
3 -C-C-;
4 -C=C-,
5 5 a heteroatom selected from the group consisting of nitrogen, oxygen,
and sulfur; or
6 -S(O)_m - .

R¹ is

- 1 C1-10 alkyl,
10 2 C2-10alkenyl,
3 C2-10 alkynyl,
4 Cy,
5 C1-10 alkyl-Cy,
6 C2-10 alkenyl-Cy, or
15 7 C2-C10 alkynyl-Cy,

wherein alkyl, alkenyl, and alkynyl are optionally substituted with one to four substituents independently selected from R^a; and Cy is optionally substituted with one to four substituents independently selected from R^b;

R² is

- 20 1 hydrogen,
2 C1-10 alkyl,
3 C2-10 alkenyl,
4 C2-10 alkynyl,
5 aryl,
25 6 C1-10 alkyl-aryl,
7 heteroaryl, or
8 C1-10 alkyl- heteroaryl,

wherein alkyl, alkenyl, and alkynyl are optionally substituted with one to four substituents independently selected from R^a, and aryl and heteroaryl are
30 optionally substituted with one to four substituents independently selected from R^b;

R³ is

1. hydrogen
2. C1-10 alkyl,

3. C2-10 alkenyl,
4. C2-10 alkynyl,
5. Cy,
6. C1-10 alkyl-Cy,
- 5 7. C2-10 alkenyl-Cy or
8. C2-10 alkynyl-Cy

Wherein alkyl, alkenyl and alkynyl are optionally substituted with one to four substituents selected from phenyl and R^x , and phenyl and Cy are optionally substituted with one to four substituents independently selected from R^y

10 R^4 is

1. hydrogen
2. C1-10 alkyl,
3. C2-10 alkenyl,
4. C2-10 alkynyl,
- 15 5. Cy,
6. C1-10 alkyl-Cy,
7. C2-10 alkenyl-Cy or
8. C2-10 alkynyl-Cy

Wherein alkyl, alkenyl and alkynyl are optionally substituted with one to four substituents selected from phenyl and R^x , and phenyl and Cy are optionally substituted with one to four substituents independently selected from R^y

20

Or R^4 with either R^f or R^g forms a mono- or bi-cyclic ring containing 0-2 heteroatoms selected from nitrogen, oxygen or sulfur, wherein nitrogen is optionally substituted with R^j , $C(O)R^e$, SO_2R^e or $SO_2NR^dR^e$

25 R^6 , R^7 , and R^8 are each independently selected from the group consisting of R^d and R^x or two of R^6 , R^7 , and R^8 and the atom to which both are attached, or two of R^6 , R^7 , and R^8 and the two adjacent atoms to which they are attached, together form a 5-7 membered saturated or unsaturated monocyclic ring containing zero to three heteroatoms selected from N, O or S,

30 R^{12} is

1. hydrogen
2. C1-10 alkyl,
3. C2-10 alkenyl,
4. C2-10 alkynyl,

5. Cy,
6. C1-10 alkyl-Cy,
7. C2-10 alkenyl-Cy or
8. C2-10 alkynyl-Cy

5 wherein alkyl, alkenyl and alkynyl are optionally substituted with one to four substituents selected from phenyl and R^x , and Cy is optionally substituted with one to four substituents independently selected from R^y

R^{13} is

- 1 hydrogen,
- 10 2 C1-10 alkyl,
- 3 C2-10 alkenyl,
- 4 C2-10 alkynyl,
- 5 aryl,
- 6 C1-10 alkyl-aryl,
- 15 7 heteroaryl,
- 8 C1-10 alkyl-heteroaryl,

wherein alkyl, alkenyl and alkynyl are optionally substituted with one to four substituents selected from R^x and aryl and heteroaryl are optionally substituted with one to four substituents independently selected from R^y ; or

20 R^{12} , R^{13} and the carbon to which they are attached form a 3-7 membered mono- or bicyclic ring containing 0-2 heteroatoms selected from N, O and S; wherein nitrogen is optionally substituted with R^j , $C(O)R^c$, SO_2R^c or $SO_2NR^dR^c$

R^{16} is selected from a bond, NR^j , O and $S(O)_n$

R^a is

- 25 1 Cy, or
- 2 a group selected from R^x ;

wherein Cy is optionally substituted with one to four substituents independently selected from R^c ;

R^b is

- 30 1 a group selected from R^a ,
- 2 C1-10 alkyl,
- 3 C2-10 alkenyl,
- 4 C2-10 alkynyl, or
- 5 C1-10 alkyl-aryl,

wherein alkyl, alkenyl, alkynyl, aryl, heteroaryl are optionally substituted with a group independently selected from R^c ;

R^c is

- 1 halogen,
- 5 2 NO₂,
- 3 C(O)OR^f
- 4 Cl-4 alkyl,
- 5 Cl-4 alkoxy,
- 6 aryl,
- 10 7 aryl Cl-4 alkyl,
- 8 aryloxy,
- 9 heteroaryl,
- 10 N R^f R^g,
- 11 N R^f C(O)R^g,
- 15 12 N R^f C(O)NR^fR^g,
- 13 CN,
- 14 C(O)Cy or
- 15 C(O)alkyl;
- 16 C(O)NR^fR^g
- 20 17 alkyloxy, or
- 18 NR^fC(O)OR^c

wherein aryl, heteroaryl and Cy are optionally substituted with 1 to 4 substituents independently selected from R^x

R^d and R^e are independently selected from hydrogen, Cl-10 alkyl, C2-10 alkenyl, C2-10 alkynyl, Cy and Cy Cl-10 alkyl, aryl, heteroaryl, aryl-substituted aryl, aryl substituted heteroaryl, heteroaryl-substituted heteroaryl wherein alkyl, alkenyl, alkynyl, heteroaryl and Cy is optionally substituted with one to four substituents independently selected from R^c ; or R^d and R^e together with the atoms to which they are attached form a heterocyclic ring of 5 to 7 members containing 0-2 additional heteroatoms independently selected from oxygen, sulfur and nitrogen; wherein nitrogen is optionally substituted with R^j, C(O)R^c, SO₂R^c or SO₂NR^dR^e

R^f and R^g are independently selected from R³ or R^f and R^g together with the carbon to which they are attached form a ring of 5 to 7 members containing 0-2 heteroatoms

independently selected from oxygen, sulfur and nitrogen wherein nitrogen is optionally substituted with R^j , $C(O)R^c$, SO_2R^c or $SO_2NR^dR^c$

R^i is

- 1 C1-10 alkyl,
- 5 2 C2-10 alkenyl,
- 3 C2-10 alkynyl, or
- 4 aryl;

wherein alkyl, alkenyl, alkynyl and aryl are each optionally substituted with one to four substituents independently selected from R^c ;

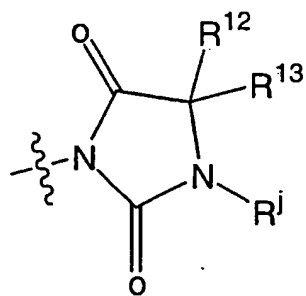
- 10 R^j is selected from hydrogen, C1-10 alkyl, C2-10 alkenyl, C2-10 alkynyl, Cy and Cy C1-10 alkyl, aryl, heteroaryl, aryl-substituted aryl, aryl substituted heteroaryl, heteroaryl-substituted heteroaryl wherein said alkyl, alkenyl, alkynyl, heteroaryl, and Cy is optionally substituted with one to four substituents independently selected from R^c

- 15 R^k and R^m are independently selected from hydrogen, C1-10 alkyl, C2-10 alkenyl, C2-10 alkynyl, Cy and Cy C1-10 alkyl, aryl, and heteroaryl

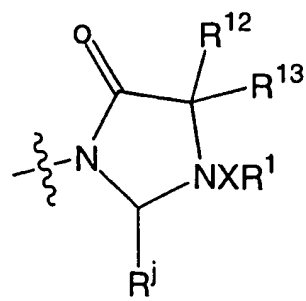
R^x is selected from

- 1 $-OR^d$,
- 2 $-NO_2$,
- 20 3 halogen,
- 4 $-S(O)_m R^d$
- 5 $-SR^d$,
- 6 $-S(O)_2OR^d$,
- 7 $-S(O)_m NR^d R^c$,
- 25 8 $-NR^d R^c$,
- 9 $-O(CR^f R^g)_n NR^d R^c$,
- 10 $-C(O)R^d$,
- 11 $-CO_2R^d$,
- 12 $-CO_2(CR^f R^g)_n CONR^d R^c$,
- 30 13 $-OC(O)R^d$,
- 14 $-CN$,
- 15 $-C(O)NR^d R^c$,
- 16 $-NR^d C(O)R^c$,
- 17 $-OC(O)NR^d R^c$,

- 18 $-N R^d C(O)O R^e$,
 19 $-N R^d C(O)N R^d R^e$,
 20 $-C R^d (N-O R^e)$,
 21 $-CF_3$,
 22 oxo,
 23 $N R^d C(O)N R^d SO_2 R^i$,
 24 $N R^d S(O)_m R^e$,
 25 $-OS(O)_2 O R^d$,
 26 $-OP(O)(O R^d)_2$;



- 28 $-N R^d C(S)N R^d R^e$, or



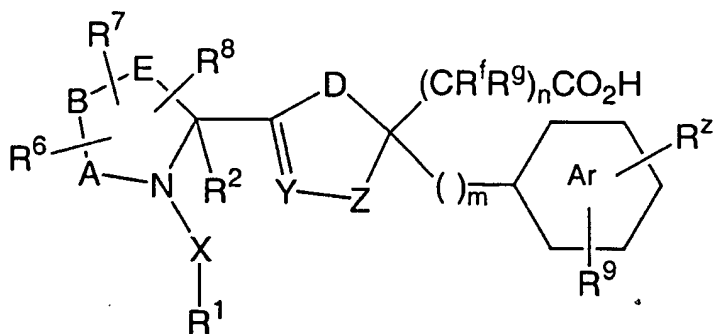
R^y is

- 1 a group selected from R^x ,
- 2 C1-10 alkyl,
- 3 C2-10 alkenyl,
- 4 C2-10 alkynyl,
- 5 aryl C1-10 alkyl- aryl,
- 6 C1-10 alkyl- heteroaryl,
- 7 cycloalkyl,
- 8 heterocyclyl
- 9 aryl
- 10 heteroaryl

wherein alkyl, alkenyl, alkynyl, heteroaryl and aryl are each optionally substituted with one to four substituents independently selected from R^x ;

Cy is cycloalkyl, heterocyclyl, aryl, or heteroaryl;

A further embodiment of the invention is the compound of the Formula II below:



where Ar is aryl, heteroaryl, aryl-substituted aryl, aryl substituted heteroaryl which are substituted with R^Z and R^9 .

R^9 is selected from H and R^y

R^Z is selected from

- 1 OR^d
- 2 NHR^d



and all other designations are as given above for the first formula.

5 These antagonists are useful in the treatment, prevention and suppression of diseases mediated by any integrin. Such diseases include multiple sclerosis, asthma, allergic rhinitis, allergic conjunctivitis, inflammatory lung diseases, rheumatoid arthritis, septic arthritis, type I diabetes, organ transplantation, restenosis, autologous bone marrow transplantation, inflammatory sequelae of viral infections, myocarditis, 10 inflammatory bowel disease including ulcerative colitis and Crohn's disease, certain types of toxic and immune-based nephritis, contact dermal hypersensitivity, psoriasis, tumor metastasis, atherosclerosis and fibrotic diseases.

DETAILED DESCRIPTION OF THE INVENTION

The present compounds are biologically active small molecules and are 15 generally composed of several domains: a) an acyl (including sulfonyl) moiety and a heterocycle #1 or a substituted aromatic ring, b) a heterocycle #2, and c) acid and a sidechain, and are named in a manner similar to that used to name oligopeptides.

Definitions:

20 An integrin "antagonist" includes any compound that inhibits a "plurality" (defined below) of integrins from binding with an integrin ligand and/or ligand receptors. For the purposes of the invention, an integrin "antagonist" also refers to agents claimed herein which can inhibit or block integrin and/or integrin ligand-mediated binding or which can otherwise modulate integrin and/or integrin ligand function, e.g., by inhibiting or blocking integrin-ligand 25 mediated integrin signal transduction. Such an antagonist of the integrin/integrin ligand interaction is an agent which has one or more of the following properties: (1) it coats, or binds to, a plurality of integrins (e.g., $\alpha 4\beta 7$, VLA-1, VLA-9 and VLA-1) on the surface of such integrin bearing or secreting cell with sufficient specificity to inhibit an integrin ligand/integrin interaction, e.g., the collagen/VLA-1 interaction; (2) it coats, or binds to, a plurality of 30 integrins on the surface of an integrin-bearing or secreting cell with sufficient specificity to modify, and preferably to inhibit, transduction of an integrin-mediated signal e.g., collagen/VLA-1-mediated signaling; (3) it coats, or binds to, a plurality of integrin receptors, (e.g., collagen only or collagen and VCAM-1) in or on cells with sufficient specificity to inhibit the integrin/integrin ligand interaction; (4) it coats, or binds to, an integrin ligand (e.g.,

collagen) in or on cells with sufficient specificity to modify, and preferably to inhibit, transduction of integrin-mediated integrin signaling, e.g., collagen-mediated VLA-1 signaling.

In preferred embodiments the integrin antagonist has one or both of properties 1 and 2. In other preferred embodiments the antagonist has one or both of properties 3 and 4. Moreover, more than one antagonist can be administered to a patient, e.g., an agent which binds to an integrin can be combined with an agent which binds to its ligand. An antagonist of the invention has "biological activity" if it inhibits a plurality of integrins from binding with an integrin ligand and/or integrin receptor as determined by in vitro and in vivo tests known to workers having ordinary skill in the art.

A "pan- β 1 antagonist" includes any compound that inhibits a plurality (defined below) of integrins containing the β 1 subunit from binding with an integrin ligand and/or receptor such as any receptor for the β 1 subunit. For the purposes of the invention only, we specifically include the integrin α 4 β 7 under the definition of a ' β 1 subunit' containing integrin. For the purposes of the invention, a "pan- β 1 antagonist" also refers to agents claimed herein which can inhibit or block integrin and/or integrin ligand-mediated binding or which can otherwise modulate integrin and/or integrin ligand function, e.g., by inhibiting or blocking integrin-ligand mediated integrin signal transduction. Such an antagonist of the integrin/integrin ligand interaction is an agent which has one or more of the properties (1) through (4) as described above. A pan- β 1 antagonist of the invention has "biological activity" if it inhibits a plurality of β 1 subunit containing integrins (including α 4 β 7) from binding with an integrin ligand and/or receptor such as any receptor for the β 1 subunit or, as defined above, any receptor for the α 4 β 7 integrin. Such biological activity is determined by in vitro and in vivo tests known to workers having ordinary skill in the art.

"Alkyl", as well as other groups having the prefix "alk", such as alkoxy, alkanoyl, means carbon chains which may be linear or branched or combinations thereof. Examples of alkyl groups include methyl, ethyl, propyl, isopropyl, butyl, sec and tert-butyl, pentyl, hexyl, heptyl, octyl, nonyl, and the like.

"Alkenyl" means carbon chains which contain at least one carbon-carbon double bond, and which may be linear or branched or combinations thereof. Examples of alkenyl include vinyl, allyl, isopropenyl, pentenyl, hexenyl, heptenyl, 1-propenyl, 2-butenyl, 2-methyl-2-butenyl, and the like.

"Alkynyl" means carbon chains which contain at least one carbon-carbon triple bond, and which may be linear or branched or combinations thereof. Examples of alkynyl include ethynyl, propargyl, 3-methyl-1-pentenyl, 2-heptynyl and the like.

"Cycloalkyl" means mono- or bicyclic saturated carbocyclic rings, each of which
5 having from 3 to 10 carbon atoms. The term also includes monocyclic rings fused to an aryl group in which the point of attachment is on the non-aromatic portion. Examples of cycloalkyl include cyclopropyl, cyclopentyl, cyclohexyl, cycloheptyl, tetrahydronaphthyl, decahydronaphthyl, indanyl, and the like.

"Aryl" means mono- or bicyclic aromatic rings containing only carbon atoms. The
10 term also includes aryl group fused to a monocyclic cycloalkyl or monocyclic heterocyclyl group in which the point of attachment is on the aromatic portion. Examples of aryl include phenyl, naphthyl, indanyl, indenyl, tetrahydronaphthyl, 2,3 dihydrobenzofuranyl, benzopyranyl, 1,4-benzodioxanyl, and the like.

"Heteroaryl" means a mono- or bicyclic aromatic ring containing at least one
15 heteroatom selected from N, O and S, with each ring containing 5 to 6 atoms. Examples of heteroaryl include pyrrolyl, isoxazolyl, isothiazolyl, pyrazolyl, pyridyl, oxazolyl, oxadiazolyl, thiadiazolyl, thiazolyl, imidazolyl, triazolyl, tetrazolyl, furanyl, triazinyl, thienyl, pyrimidyl, pyridazinyl, pyrazinyl, benzoxazolyl, benzothiazolyl, benzimidazolyl, benzofuranyl, benzothiophenyl, furo(2,3-b)pyridyl, quinolyl, indolyl, isoquinolyl, and the like.

"Heterocyclyl" means mono- or bicyclic saturated rings containing at least one
20 heteroatom selected from N, S and O, each of said ring having from 3 to 10 atoms in which the point of attachment may be carbon or nitrogen. The term also includes monocyclic heterocycle fused to an aryl or heteroaryl group in which the point of attachment is on the non-aromatic portion. Examples of "heterocyclyl" include pyrrolidinyl, piperidinyl, piperazinyl, imidazolidinyl, 2,3-dihydrofuro(2,3-b) pyridyl, benzoxazinyl, tetrahydrohydroquinolyl, tetrahydroisoquinolyl, dihydroindolyl, and the like. The term also includes partially unsaturated monocyclic rings that are not aromatic, such as 2- or 4 pyridones attached through the nitrogen or N-substituted- (1H,3H) pyrimidine-2,4-diones (N-substituted uracils).

"Halogen" includes fluorine, chlorine, bromine and iodine.

30 "Plurality" is intended to mean: (I) any single integrin; or (II) two or more integrins. Thus, the present methods utilize: (i) molecules capable of inhibiting any combination of two or more different integrins such as a molecule capable of antagonizing both VLA-4 ($\alpha 4\beta 1$) and $\alpha 4\beta 7$ or VLA-2 ($\alpha 2\beta 1$), VLA-6 ($\alpha 6\beta 1$) and VLA-4, and so on; or (ii) molecules capable of selectively inhibiting any one integrin, such as $\alpha 4\beta 7$ only or VLA-4 only. Thus a pan-beta 1

antagonist inhibits any single integrin containing a beta1 subunit (including $\alpha4\beta7$) or two or more integrins that contain a beta1 subunit (such two or more integrins which may include $\alpha4\beta7$).

“Polymer” has its art recognized meaning as being a molecule constructed from many smaller structural units called “monomers”, bonded together (preferably covalently) in any pattern. The term includes linear molecules and branched molecules. The term also includes homopolymers where only one species of monomer is used to build the molecule, or copolymers where the molecule is composed of two different types of monomers and so on. Copolymers also include polymers where the distribution of monomers is random, alternating
10 copolymers, block copolymers and graft copolymers.

Most preferably, the polymer is ‘biocompatible’. A “biocompatible” substance, as that term is used herein, is one that has no unacceptable toxic or injurious effects on biological function.

Antagonists of the invention are ‘small molecules’ which are organic molecules.
15 A “small molecule”, as defined herein, has a molecular weight generally less than 2000.

The term “effective amount” as used herein, means an amount of a compound of the present invention which inhibits a “plurality” (defined herein) of integrins from binding with an integrin ligand and/or integrin receptor, as determined by in vitro and in vivo tests known to workers having ordinary skill in the art.

20 Optical Isomers - Diastereomers - Geometric Isomers – Tautomers

Compounds of Formula I contain one or more asymmetric centers and can thus occur as racemates and racemic mixtures, single enantiomers, diastereomeric mixtures and individual diastereomers. The present invention is meant to comprehend all such isomeric forms of the compounds of Formula I.

25 Some of the compounds described herein contain olefinic double bonds, and unless specified otherwise, are meant to include both E and Z geometric isomers. Some of the compounds described herein may exist with different points of attachment of hydrogen, referred to as tautomers. Such an example may be a ketone and its enol form known as keto-enol tautomers. The individual tautomers as well as mixture thereof are encompassed with
30 compounds of Formula I.

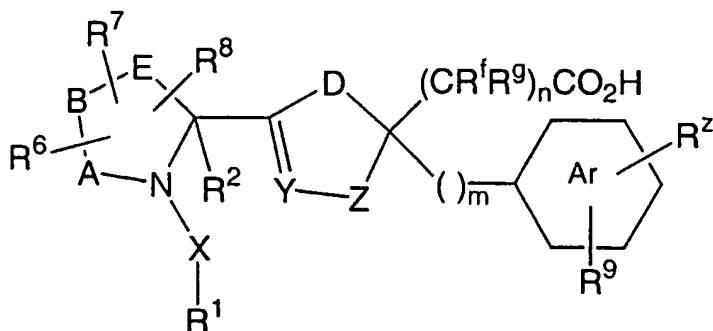
Compounds of the Formula I may be separated into diastereoisomeric pairs of enantiomers by, for example, fractional crystallization from a suitable solvent, for example methanol or ethyl acetate or a mixture thereof. The pair of enantiomers thus obtained may be separated into individual stereoisomers by conventional means, for example by the use of an

optically active acid as a resolving agent. Alternatively, any enantiomer of a compound of the general Formula I may be obtained by stereospecific synthesis using optically pure starting materials or reagents of known configuration.

Other Modifications of the Antagonists

5 Other species are within the scope of the generic formulae are described herein.

For example, an exemplary series of antagonists is found in Formula II :



10

where Ar is aryl, heteroaryl, aryl-substituted aryl, aryl substituted heteroaryl which are substituted with R^z and R⁹.

R⁹ is selected from H and R^y

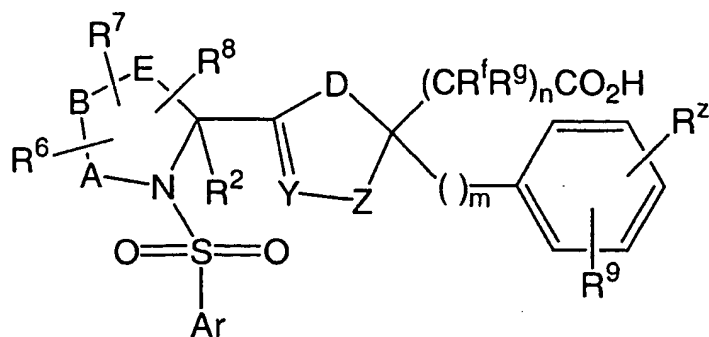
R^z is selected from

- 15
- | | |
|---|--|
| 1 | OR ^d |
| 2 | NHR ^d |
| 3 | NR ^d S(O) _m R ^c |
| 4 | NR ^d C(O)R ^c |

and all other designations and substituents are as above.

20

Another series are antagonists of Formula (III):



wherein Ar is aryl, heteroaryl, aryl-substituted aryl, aryl substituted heteroaryl which are optionally substituted with one to four substituents independently selected from R^x

5 and R⁹ is selected from H and R^y.

R^z is selected from

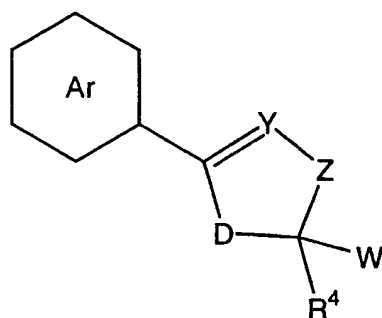
- | | |
|------|--|
| 1 | OR ^d |
| 2 | NHR ^d |
| 3 | NR ^d S(O) _m R ^e |
| 10 4 | NR ^d C(O)R ^e |

and all other designations and substituents are as previously recited as above.

15

20

Further series are those of antagonists of Formula IV:

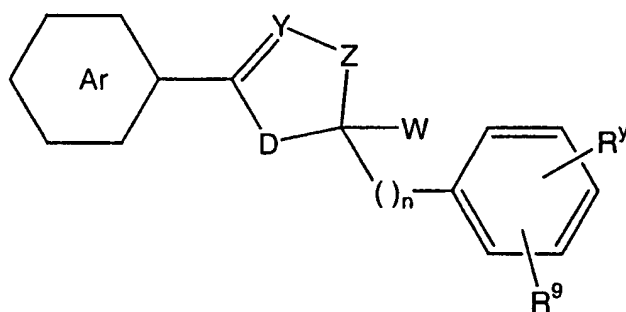


wherein Ar is selected from aryl, heteroaryl, aryl substituted aryl, aryl
 5 substituted heteroaryl, and heteroaryl substituted heteroaryl and further wherein

said aryl, heteroaryl, aryl substituted aryl, aryl substituted heteroaryl, heteroaryl
 substituted heteroaryl are optionally substituted with 1 to 4 substituents independently
 selected from R^x and all other designations and substituents are as recited above.

10

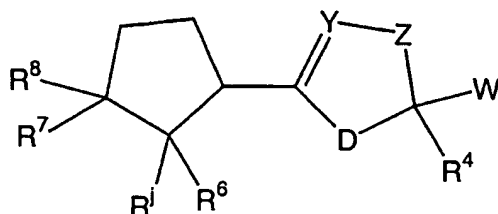
Further embodiments are found in antagonists having the formula V:



15

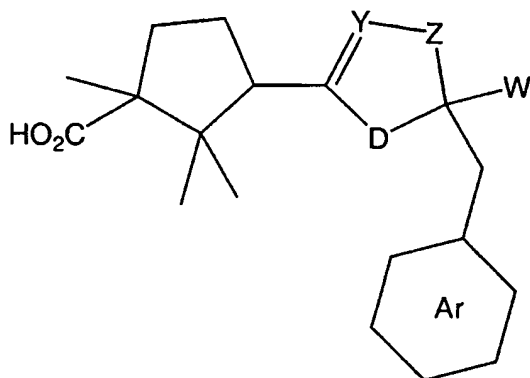
wherein Ar is selected from aryl, heteroaryl, aryl substituted aryl, aryl substituted
 heteroaryl, and heteroaryl substituted heteroaryl and further wherein said aryl,
 heteroaryl, aryl substituted aryl, aryl substituted heteroaryl, heteroaryl substituted
 20 heteroaryl are optionally substituted with 1 to 4 substituents independently selected
 from R^x , R^9 is selected from H and R^y and all other designations and substituents are as
 recited above

Other antagonists of the invention are shown below as Formula VI:



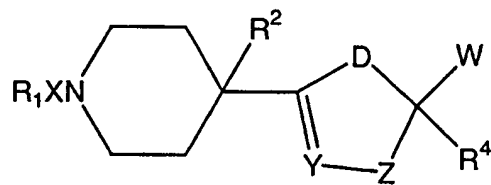
wherein all designations and substituents are as recited above.

Another series of antagonists is shown below as Formula VII:



wherein Ar is selected from aryl, heteroaryl, aryl substituted aryl, aryl substituted heteroaryl, and heteroaryl substituted heteroaryl and further wherein said aryl, heteroaryl, aryl substituted aryl, aryl substituted heteroaryl, heteroaryl substituted heteroaryl are optionally substituted with 1 to 4 substituents independently selected from R^x and all other designations and substituents are as recited above. Further series are those of Formulae VIII, IX, XI, XII, and XIII, where all designations and substituents are as recited above.

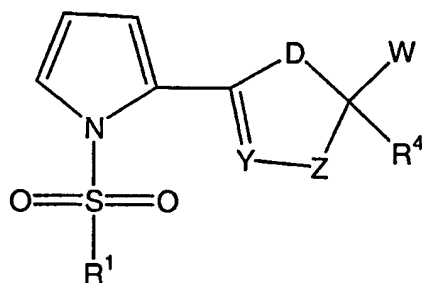
VIII



5

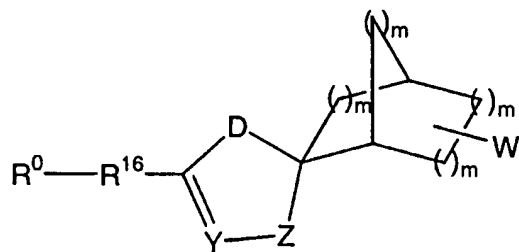
10

IX:



15

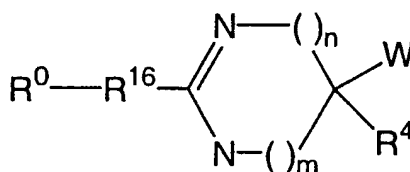
XI:



20

XII:

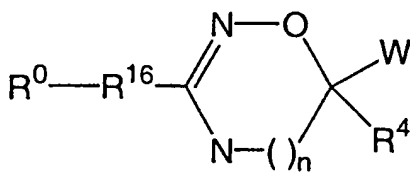
5



10

15

XIII:

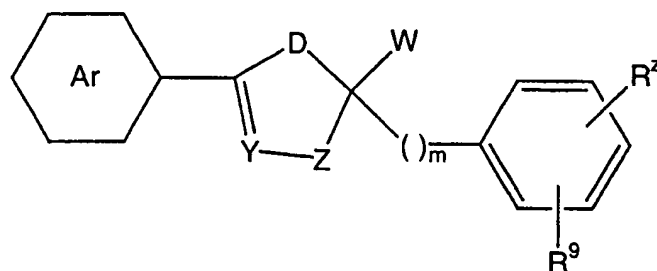


20

Yet another series of antagonists are those of Formula X:

25

X:



Ar is selected from aryl, heteroaryl, aryl substituted aryl, aryl substituted heteroaryl, and heteroaryl substituted heteroaryl and further wherein said aryl, heteroaryl, aryl substituted aryl, aryl substituted heteroaryl, heteroaryl substituted heteroaryl are optionally substituted with 1 to 4 substituents independently selected from R^x , R^y is selected from H and R^y , R^z is selected from

- 1 OR^d
- 2 NHR^d
- 10 3 $NR^dS(O)_mR^e$
- 4 $NR^dC(O)R^e$

and all other designations and substituents are as recited above.

15 Specific structures intended to fall within the scope of the present invention are analysed on either a Platform LCZ mass spectrometer (electrospray positive) or a VG Platform II mass spectrometer (electrospray positive or negative).

Polymer Conjugate Forms

20 Within the broad scope of the present invention, a single polymer molecule may be employed for conjugation with an integrin antagonist, although it is also contemplated that more than one polymer molecule can be attached as well. Conjugated integrin antagonist compositions of the invention may find utility in both *in vivo* as well as non-*in vivo* applications. Additionally, it will be recognized that the conjugating polymer may utilize any other groups, moieties, or other conjugated species, as appropriate to the end use application. By way of example, it may be useful in some applications to covalently bond to the polymer a functional moiety imparting UV-degradation resistance, or antioxidation, or other properties or characteristics to the polymer. As a further example, it may be advantageous in some applications to functionalize the polymer to render it reactive and enable it to cross-link to a drug molecule, to enhance

various properties or characteristics of the overall conjugated material. Accordingly, the polymer may contain any functionality, repeating groups, linkages, or other constituent structures which do not preclude the efficacy of the conjugated integrin antagonist composition for its intended purpose. Other objectives and advantages of the present invention will be more fully apparent from the ensuing disclosure and appended claims.

Illustrative polymers that may usefully be employed to achieve these desirable characteristics are described herein below in exemplary reaction schemes. In one embodiment of a covalently bonded antagonist/polymer conjugate, the polymer may be coupled to the antagonist to form stable bonds that are not significantly cleavable by human enzymes. Generally, for a compound of the invention to contain bonds that are not 'significantly' cleavable requires that no more than about 20% of the bonds of the compound are cleaved within a 24 hour period, as measured by standard techniques in the art including, but not limited to, high pressure liquid chromatography (HPLC).

Integrin antagonists of the invention are conjugated most preferably via a terminal reactive group on the polymer although conjugations can also be branched from non-terminal reactive groups. The polymer with the reactive group(s) is designated herein as "activated polymer". The reactive group selectively reacts with reactive groups on the antagonist molecule. The activated polymer(s) is reacted so that attachment may occur at any available integrin antagonist functional group. Amino, carbon, free carboxylic groups, suitably activated carbonyl groups, hydroxyl, guanidyl, oxidized carbohydrate moieties, amino, carbon and mercapto groups of the integrin antagonist (if available) can be used as attachment sites.

Generally from about 1.0 to about 10 moles of activated polymer per mole of antagonist, depending on antagonist concentration, is employed. The final amount is a balance between maximizing the extent of the reaction while minimizing non-specific modifications of the product and, at the same time, defining chemistries that will maintain optimum activity, while at the same time optimizing, if possible, the half-life of the antagonist. Preferably, at least about 50% of the biological activity of the antagonist is retained, and most preferably 100% is retained.

The reactions may take place by any suitable art-recognized method used for reacting biologically active materials with inert polymers. Generally the process involves preparing an activated polymer and thereafter reacting the antagonist with the activated polymer to produce the soluble compound suitable for formulation. The

above modification reaction can be performed by several methods, which may involve one or more steps.

The polymeric substances included herein are preferably water-soluble at room temperature. A non-limiting list of such polymers includes polyalkylene oxide
5 homopolymers such as polyethylene glycol (PEG) or polypropylene glycols, polyoxyethylenated polyols, copolymers thereof and block copolymers thereof, provided that the water solubility of the block copolymers is maintained.

In the preferred practice of the present invention, polyalkylene glycol residues of C1-C4 alkyl polyalkylene glycols, preferably polyethylene glycol (PEG), or
10 poly(oxy)alkylene glycol residues of such glycols are advantageously incorporated in the polymer systems of interest. Thus, the polymer to which the antagonist is attached can be a homopolymer of polyethylene glycol (PEG) or is a polyoxyethylated polyol, provided in all cases that the polymer is soluble in water at room temperature. Non-limiting examples of such polymers include polyalkylene oxide homopolymers such as
15 PEG or polypropylene glycols, polyoxyethylenated glycols, copolymers thereof and block copolymers thereof, provided that the water solubility of the block copolymer is maintained. Examples of polyoxyethylated polyols include, for example, polyoxyethylated glycerol, polyoxyethylated sorbitol, polyoxyethylated glucose, or the like. The glycerol backbone of polyoxyethylated glycerol is the same backbone
20 occurring naturally in, for example, animals and humans in mono-, di-, and triglycerides. Therefore, this branching would not necessarily be seen as a foreign agent in the body.

A general formula for PEG and its derivatives is $R''-(CH_2CH_2O)_x-(CH_2)_y-R'$, where (x) represents the degree of polymerization or number of repeating units in
25 the polymer chain and is dependent on the molecular weight of the polymer, (y) represents a positive integer, R' is (CHR^1) , where R¹ is as defined in claim 1 and R'' is a capping group (including, without limitation, OH, C[1-4]alkyl moieties, or various biologically active and inactive moieties) or is R'. In particular, polyethylene glycols (PEG's), mono-activated, C[1-4]alkyl-terminated PAO's such as mono-methyl-
30 terminated polyethylene glycols (mPEG's) are preferred when mono-substituted polymers are desired; bis-activated polyethylene oxides are preferred when disubstituted antagonists are desired.

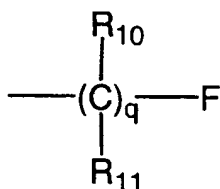
As an alternative to polyalkylene oxides, dextran, polyvinyl pyrrolidones, polyacrylamides, polyvinyl alcohols, carbohydrate-based polymers and the like may be used.

Those of ordinary skill in the art will recognize that the foregoing list is merely illustrative and that all polymer materials having the qualities described herein are contemplated. The polymer need not have any particular molecular weight, but it is preferred that the molecular weight be between about 300 and 100,000, more preferably between 10,000 and 40,000. In particular, sizes of 20,000 or more are best at preventing loss of the product due to filtration in the kidneys.

Polyalkylene glycol derivatization has a number of advantageous properties in the formulation of polymer- integrin antagonist conjugates in the practice of the present invention, as associated with the following properties of polyalkylene glycol derivatives: improvement of aqueous solubility, while at the same time eliciting no antigenic or immunogenic response; high degrees of biocompatibility; absence of in vivo biodegradation of the polyalkylene glycol derivatives; and ease of excretion by living organisms.

Polyethylene glycol (PEG) and related polyalkylene oxides (PAO's) are known in the art as being useful adjuncts for the preparation of drugs. See for example, PCT WO 93/24476. PEG has also been conjugated to proteins, peptides and enzymes to increase aqueous solubility and circulating life in vivo as well as reduce antigenicity. See, for example, U.S. Pat. Nos. 5,298,643 and 5,321,095, both to Greenwald, et al. PCT WO 93/24476 discloses using an ester linkage to covalently bind an organic molecule to water-soluble polyethylene glycols.

In one aspect of the invention, one can utilize an integrin antagonist covalently bonded to the polymer component in which the nature of the conjugation involves one or more noncleavable covalent chemical bonds which, preferably, are resistant to degradation by human enzymes. For instance, Greenwald et al., supra, disclose biologically-active conjugates having substantially hydrolysis-resistant bonds (linkages) between a polyalkylene oxide and the target moiety. One example of a noncleavable linker suitable for the antagonists of the present invention is:



wherein R_{10} and R_{11} are independently selected from the group consisting of H, C_{1-6} alkyls, aryls, substituted aryls, aralkyls, heteroalkyls, substituted heteroalkyls and substituted C_{1-6} alkyls, q is a positive integer and F is selected from O, NR^1 , S, SO, SO_2 .

In another embodiment, the linkages between a polymer and the antagonist of the invention is cleavable, allowing for control in terms of the time course over which the polymer may be cleaved from the integrin antagonist. This covalent bond between the integrin antagonist and the polymer may be cleaved by chemical or enzymatic reaction. In order to provide a hydrolyzable linkage, mono- or di-acid activated polymers such as PEG acids or PEG diacids are used. Suitable PAO acids can be synthesized by converting mPEG-OH to an ethyl ester. See also Gehrhardt, H., et al. Polymer Bulletin 18: 487 (1987) and Veronese, F. M., et al., J. Controlled Release 10: 145 (1989). Alternatively, the PAO-acid can be synthesized by converting mPEG-OH into a t-butyl ester. Ohya, et al., J. Bioactive and Compatible Polymers Vol. 10 Jan., 1995, 51-66, disclose doxorubicin-PEG conjugates which are prepared by linking the two substituents via various linkages including esters. It will be clear from the foregoing that other polyalkylene oxide derivatives of the foregoing, such as the polypropylene glycol acids, POG acids, etc., as well as other bifunctional linking groups are also contemplated. The polymer- integrin antagonist product retains an acceptable amount of activity. Concurrently, portions of polyethylene glycol are present in the conjugating polymer to endow the polymer- integrin antagonist conjugate with high aqueous solubility and prolonged blood circulation capability.

It is to be understood that the reaction schemes described herein are provided for the purposes of illustration only and are not to be limiting with respect to the reactions and structures which may be utilized in the modification of the integrin antagonist, e.g., to achieve solubility, stabilization, and cell membrane affinity for parenteral and oral administration. The activity and stability of the integrin antagonist conjugates can be varied in several ways by using a polymer of different molecular size. Solubilities of the conjugates can be varied by changing the proportion and size of the polyethylene glycol fragment incorporated in the polymer composition.

Salts

The term "pharmaceutically acceptable salts" refers to salts prepared from pharmaceutically acceptable non-toxic bases or acids including inorganic or organic bases and inorganic or organic acids. Salts derived from inorganic bases include aluminum, ammonium, calcium, copper, ferric, ferrous, lithium, magnesium, manganic salts, manganous, potassium, sodium, zinc, and the like. Particularly preferred are the ammonium, calcium, magnesium, potassium, and sodium salts. Salts derived from pharmaceutically acceptable organic non-toxic bases include salts of primary, secondary, and tertiary amines, substituted amines including naturally occurring substituted amines, cyclic amines, and basic ion exchange resins, such as arginine, betaine, caffeine, choline, N, N'-dibenzylethylenediamine, diethylamine, 2-diethylaminoethanol, 2-dimethylaminoethanol, ethanolamine, ethylenediamine, N-ethylmorpholine, N-ethylpiperidine, glucamine, glucosamine, histidine, isopropylamine, lysine, methylglucamine, morpholine, piperazine, piperidine, polyamine resins, procaine, purines, theobromine, triethylamine, trimethylamine, tripropylamine, tromethamine, and the like.

When the compound of the present invention is basic, salts may be prepared from pharmaceutically acceptable non-toxic acids, including inorganic and organic acids. Such acids include acetic, benzenesulfonic, benzoic, camphorsulfonic, citric, ethanesulfonic, fumaric, gluconic, glutamic, hydrobromic, hydrochloric, isethionic, lactic, maleic, malic, mandelic, methanesulfonic, mucic, nitric, pantoic, pantothenic, phosphoric, succinic, sulfuric, tartaric, p-toluenesulfonic acid, and the like. Particularly preferred are citric, hydrobromic, hydrochloric, maleic, phosphoric, sulfuric, and tartaric acids.

It will be understood that, as used herein, references to the compounds of Formula I are meant to also include the pharmaceutically acceptable salts.

Utilities

The ability of the compounds of Formula I to antagonize the actions of any VLA integrin containing a $\beta 1$ subunit (including $\alpha 4 \beta 7$) makes them useful for preventing or reversing the symptoms, disorders or diseases induced by the binding of VLA to its various ligands. Thus, these antagonists will inhibit cell adhesion processes including cell activation, migration, proliferation and differentiation and be useful in conditions such as acute or chronic renal failure or acute brain injury. Accordingly, another aspect of the present invention provides a method for the treatment (including prevention, alleviation, amelioration or suppression) of diseases or disorders or symptoms, including fibrotic conditions and an inflammatory disorder mediated by

integrin binding and cell adhesion activation, which comprises administering to a mammal an effective amount of a compound of Formula I.

As used herein, "an inflammatory disorder", includes, but is not limited to, skin related conditions such as psoriasis, eczema, burns and dermatitis. Other inflammatory disorders contemplated for treatment by the methods of the present invention include, but are not limited to the treatment of asthma, bronchitis, menstrual cramps, tendinitis, bursitis, and the treatment of pain and headaches, or as an antipyretic for the treatment of fever. The methods of the invention also would be useful to treat gastrointestinal conditions such as inflammatory bowel disease, Crohn's disease, gastritis, irritable bowel syndrome and ulcerative colitis and for the prevention of colorectal cancer. The methods of the invention would be useful in treating inflammation in such diseases as vascular diseases, migraine headaches, periarteritis nodosa, thyroiditis, aplastic anemia, Hodgkin's disease, rheumatic fever, type I diabetes, myasthenia gravis, multiple sclerosis, sarcoidosis, nephrotic syndrome, Behcet's syndrome, polymyositis, gingivitis, hypersensitivity, conjunctivitis, swelling occurring after injury, myocardial ischemia, and the like. The methods of the invention would also be useful in the treatment of allergic rhinitis, respiratory distress syndrome, endotoxin shock syndrome, and atherosclerosis as well as asthma, allergic rhinitis, allergic conjunctivitis, inflammatory lung diseases, rheumatoid arthritis, septic arthritis, organ transplantation rejection, restenosis, autologous bone marrow transplantation, inflammatory sequelae of viral infections, myocarditis, tumor metastasis and atherosclerosis.

Antagonists of the present invention may also be useful in treating a subject with a fibrotic condition. The term "fibrotic condition" refers to, but is not limited to, subjects afflicted with fibrosis of an internal organ, subjects afflicted with a dermal fibrosing disorder, and subjects afflicted with fibrotic conditions of the eye.

Fibrosis of internal organs (e.g., liver, lung, kidney, heart blood vessels, gastrointestinal tract) occurs in disorders such as pulmonary fibrosis, myelofibrosis, liver cirrhosis, mesangial proliferative glomerulonephritis, crescentic glomerulonephritis, diabetic nephropathy, renal interstitial fibrosis, renal fibrosis in patients receiving cyclosporin, and HIV associated nephropathy. Dermal fibrosing disorders include, but are not limited to, scleroderma, morphea, keloids, hypertrophic scars, familial cutaneous collagenoma, and connective tissue nevi of the collagen type. Fibrotic conditions of the eye include conditions such as diabetic retinopathy, postsurgical scarring (for example, after glaucoma filtering surgery and after cross-eye

surgery), and proliferative vitreoretinopathy. Additional fibrotic conditions which may be treated by the methods of the present invention include: rheumatoid arthritis, diseases associated with prolonged joint pain and deteriorated joints; progressive systemic sclerosis, polymyositis, dermatomyositis, eosinophilic fascitis, morphea, Raynaud's syndrome, and nasal polyposis.

In addition, fibrotic conditions which may be treated the methods of present invention also include inhibiting overproduction of scarring in patients who are known to form keloids or hypertrophic scars, inhibiting or preventing scarring or overproduction of scarring during healing of various types of wounds including surgical incisions, surgical abdominal wounds and traumatic lacerations, preventing or inhibiting scarring and reclosing of arteries following coronary angioplasty, preventing or inhibiting excess scar or fibrous tissue formation associated with cardiac fibrosis after infarction and in hypersensitive vasculopathy.

Testing Antagonists of the Invention for Function

IN VITRO TESTING

The cell adhesion inhibitory activity of these compounds may be measured by determining the concentration of inhibitor required to block the binding of cells expressing integrins to extracellular matrix components such as collagen or fibronectin coated plates. In this assay microtiter wells are coated with, for example, collagen. Once the wells are coated, varying concentrations of the test compound are then added together with appropriately labeled, integrin-expressing cells. Alternatively, the test compound may be added first and allowed to incubate with the coated wells prior to the addition of the cells. The cells are allowed to incubate in the wells for at least 30 minutes. Following incubation, the wells are emptied and washed. Inhibition of binding is measured by quantitating the fluorescence or radioactivity bound to the plate for each of the various concentrations of test compound, as well as for controls containing no test compound.

Integrin expressing cells that may be utilized in this assay include Ramos cells, Jurkat cells, A375 melanoma cells, as well as human peripheral blood lymphocytes (PBLs). These cells are commercially available and may be fluorescently or radioactively labeled if desired. A direct binding assay may also be employed to quantitate the inhibitory activity of the compounds of this invention. ("DBA").

Generally, in vitro assays such as the adhesion inhibition and direct binding assays described above, substitute the appropriate integrin-expressing cell and

corresponding ligand. For example, polymorphonuclear cells (PMNs) express integrins on their surface and bind to ICAM. Integrins are involved in platelet aggregation and inhibition may be measured in a standard platelet aggregation assay. VLA-5 binds specifically to Arg-Gly-Asp sequences, while VLA-6 binds to laminin.

5 IN VIVO TESTING

Once antagonists are identified, they may be further characterized in in vivo assays, non-limiting examples of which are described below:

A. Contact Hypersensitivity

An exemplary animal model is described by P.L. Chisholm et al., "Monoclonal
10 Antibodies to the Integrin α -4 Subunit Inhibit the Murine Contact Hypersensitivity Response", Eur. J. Immunol., 23, pp. 682-688 (1993) and in "Current Protocols in Immunology", J. E. Coligan, et al., Eds., John Wiley & Sons, New York, 1, pp. 4.2.1-4.2.5 (1991), the disclosures of which are herein incorporated by reference. In these assays, the skin of the animal is sensitized by exposure to an irritant, such as
15 dinitrofluorobenzene, followed by light physical irritation, such as scratching the skin lightly with a sharp edge. Following a recovery period, the animals are re-sensitized following the same procedure. Several days after sensitization, one ear of the animal is exposed to the chemical irritant, while the other ear is treated with a non-irritant control solution. Shortly after treating the ears, the animals are given various doses of the
20 antagonists by subcutaneous injection. In vivo inhibition of cell adhesion-associated inflammation is assessed by measuring the ear swelling response of the animal in the treated versus untreated ear. Swelling is measured using calipers or other suitable instrument to measure ear thickness.

B. Delayed hypersensitivity

25 SRBC-induced delayed type hypersensitivity (DTH) responses are adapted from the protocol of Hurtrel et al. 1992 Cell. Immunol. 142:252-263. Briefly, mice are immunized s.c. in the back with 2×10^7 SRBC in 100 μ l PBS on d 0. The mice are challenged on d 5 by injecting 1×10^8 SRBC in 25 μ l PBS s.c into the right hind footpad. Footpad thickness is measured with an engineer's caliper 20 h after antigen
30 challenge, and the degree of footpad swelling calculated. Results are reported as the mean percent increase footpad thickness \pm SEM and calculated as % increase = $[1 - (\text{Right footpad thickness 20 h after antigen challenge} / \text{Uninjected left footpad thickness 20 h after antigen challenge})] \times 100$. To block the effector phase of the SRBC-induced DTH response, antagonists of the invention which are prepared according to the

methods described in the Examples is given prior to antigen challenge on d 5. SRBC-induced DTH is a well characterized *in vivo* model of inflammation, and in particular psoriasis, that has been used to demonstrate the importance of a variety of cytokines and adhesion molecules in inflammation (Tedder et al. 1995 *J. Exp. Med.* 181:2259-2264, Terashita et al. 1996 *J. Immunol.* 156:4638-4643).

In this manner, one may identify those inhibitors of this invention which are best suited for inhibiting inflammation.

C. Asthma

Another *in vivo* assay that may be employed to test the antagonists of this invention is the sheep asthma assay. This assay is performed essentially as described in W. M. Abraham et al., " α -Integrins Mediate Antigen-induced Late Bronchial Responses and Prolonged Airway Hyperresponsiveness in Sheep", *J. Clin. Invest.*, 93, pp. 776-87 (1994), the disclosure of which is herein incorporated by reference. This assay measures inhibition of *Ascaris* antigen-induced late phase airway responses and airway hyperresponsiveness in allergic sheep

D. Renal Failure

The agents of the present invention also may be tested in animal models of chronic renal failure. Mammalian models of chronic renal failure in, for example, mice, rats, guinea pigs, cats, dogs, sheep, goats, pigs, cows, horses, and non-human primates, may be created by causing an appropriate direct or indirect injury or insult to the renal tissues of the animal. Animal models of chronic renal failure may, for example, be created by performing a partial (e.g., 5/6) nephrectomy which reduces the number of functioning nephron units to a level which initiates compensatory renal hypertrophy, further nephron loss, and the progressive decline in renal function which characterizes chronic renal failure. The agents of the present invention may be evaluated for their therapeutic efficacy in causing a clinically significant improvement in a standard marker of renal function when administered to a mammalian subject (e.g., a human patient) in, or at risk of, chronic renal failure. Such markers of renal function are well known in the medical literature and include, without being limited to, rates of increase in BUN levels, rates of increase in serum creatinine, static measurements of BUN, static measurements of serum creatinine, glomerular filtration rates (GFR), ratios of BUN/creatinine, serum concentrations of sodium (Na⁺), urine/plasma ratios for creatinine, urine/plasma ratios for urea, urine osmolality, daily urine output, and the like (see, for example, Brenner and Lazarus (1994), in Harrison's Principles of Internal

Medicine, 13th edition, Isselbacher et al., eds., McGraw Hill Text, New York; Luke and Strom (1994), in Internal Medicine, 4th Edition, J.H. Stein, ed., Mosby-Year Book, Inc. St. Louis.).

E. Acute Brain Injury

5 Male Sprague Dawley (SD) or spontaneously hypertensive rats (SHRS) are anesthetized using isoflurane and the right middle cerebral artery (MCAO) occluded by insertion of a 4-0 nylon monofilament up the internal carotid artery to the origin of the middle cerebral artery (MCA) (Zea Longa et al, 1989 *Stroke* 20:84). After 1h the filament is retracted, the ischemic territory reperfused and the animal allowed to
10 recover. After 24h the rats are sacrificed, at which time brains were removed and analyzed histologically to quantify infarct volume.

Groups of animals are treated with either vehicle (PBS) or antagonist of the invention by continuous subcutaneous infusion via osmotic mini-pumps. Primed mini osmotic pumps (for example from Alza Corp.) are implanted into the subcutaneous
15 space at the scruff of the neck immediately prior to induction of cerebral ischemia. The pumps are loaded to release antagonist.

F. Fibrosis

For vessel injury leading to fibrosis, male Sprague-Dawley rats weighing 400g and about 3-4 months of age (Bantin & Kingman, Edwards, WA) are used. The left
20 common carotid artery is denuded with a 2F balloon catheter by introducing the catheter through the external carotid artery. The distal left common carotid and external carotid arteries are exposed through a midline wound in the neck. The catheter is passed three times with the balloon distended sufficiently with saline to generate slight resistance; this method produces distension of the carotid itself. the external
25 carotid is ligated after removal of the catheter and the wound closed. Experimental treatments include a series of injections of antagonist given every other day (post-operation). After 14 days post balloon catheter denudation, all rats are anesthetized and the carotid arteries fixed by perfusion at 120 mm Hg pressure with 1% paraformaldehyde and 1.25% glutaraldehyde in 0.1M phosphate buffer, pH 7.4 via a
30 large cannula placed retrograde in the abdominal aorta. Ten minutes before fixation, these animals are given an intravenous injection of Evans blue (0.3 ml in 5% saline solution). After 5 minutes of perfusion, the entire left and right common carotid arteries are retrieved, including the aortic arch. The vessels are further fixed by immersion in the same fixative as was used for perfusion. Arterial segments are

assayed for the presence or absence of endothelium by obtaining three segments from the denuded, blue-stained left carotid artery and embedding them in paraffin for cross sectioning using a microtome. For measuring intimal areas, photomicrographs are obtained from 3-4 sections from each animal. The photomicrographs are digitized and
5 analysed with image analysis software (such as NIH Image 1.55 for MacIntosh). Intimal areas are measured by determining the area between lumen and internal elastic lamina. Medial areas are determined by measuring the area between internal and external elastic lamina. Intimal/medial area ratios are calculated from the measurements.

10 For testing the effect of the present antagonists on lung fibrosis, chronic respiratory disease-free Golden Syrian hamster weighing 120-130g are purchased (e.g., from Charles River, Boston, MA) and housed in plastic cages in groups of 4 in facilities approved by the American Association for Accreditation of Laboratory Animal Care. The animals are allowed to acclimate for one week to laboratory conditions prior to
15 starting the experiments. They have access to food and water *ad libitum* and housed in a room which gets the filtered air and has 12hr/12hr light/dark cycle. Bleomycin sulfate is dissolved in pyrogen free sterile isotonic saline just before intratracheal (IT) instillation. Under pentobarbital anesthesia (25-35mg/kg ip) hamsters in appropriate group receive either bleomycin (5.5 units/kg/4ml) or an equivalent volume (4ml/kg) of
20 pyrogen free isotonic saline through transoral route. The antagonists of the invention are administered by intraperitoneal (IP) or intratracheal route at a therapeutic dose to hamster in appropriate groups twice a week for 21-28 days post installation. Thereafter, the animals in each group are killed by an overdose of sodium pentobarbital (100-125 mg/kg ip) and their lungs processed for biochemical and histopathological
25 studies.

G. Glomerulonephritis Model

The experimental lesion is acute mesangial proliferative glomerulonephritis and is characterized by expansion of the mesangial matrix and hypercellularity. Of particular interest, the nephritis reproducibly progresses through glomerular and
30 tubulointerstitial scarring, to end stage renal disease.

First, glomerulonephritis is induced in rats with a single injection of anti-glomerular basement membrane nephrotoxin serum (NTS), derived in rabbits. Next, for six days, two groups of rats are treated with daily intravenous injections of saline (the negative control group) or antagonists of the invention. On the tenth day, the animals

are sacrificed and slides are made of the kidneys, which are stained with periodic acid-Schiff solution to emphasize the pathological changes. The extent of glomerular injury can be quantitated by performing glomerular cell counts from 30 randomly selected glomeruli from normal animals and nephritic animals in each group. Another measure
5 of the effect of antagonists of the invention on the disease process is to quantitate the amount of extracellular matrix accumulation in the glomeruli. The degree of glomerular matrix expansion is determined as the percentage of each glomerulus occupied by the mesangial matrix according to the method of Raij et al. (1984) *Kidney Int.* 26: 137-43.

H. Arthritis Model

10 Arthritis is induced in pathogen-free female LEW rats (Harlan Sprague Dawley, Indianapolis, Ind.) weighing about 100 grams. Each receives a dose of cell wall fragments from Group A streptococci (SCW) (30 μ g rhamnose/gm bodyweight), injected intraperitoneally (ip) according to the technique described in Brandes et al. (1991) *J. Clin. Invest.* 87:1108. SCW-injected and control LEW rats are given an
15 intraarticular (IA) injection in one of the hind ankles of antagonists of the invention, carrier only, or a control.

Joints are clinically monitored by determining the amount of joint erythema, swelling and distortion on a scale of 0 (normal) to 4 (severe inflammation). Radiographs are taken and are evaluated for soft tissue swelling, joint space narrowing,
20 bone erosions and deformity. Tissue specimens are obtained and prepared for histopathologic analysis as described in Brandes et al., *ibid.* Total RNA is isolated from excised synovial tissues according to the method of Allen et al. (1990) *J. Exp. Med.* 171:231.

Other models are available. See Terato et al. 1992 *J. Immunol.* 148:2103-2108;
25 Terato et al. 1995 *Autoimmunity*. 22:137-147. Briefly, arthritis is induced through i.p. injection of a cocktail of 4 anti-collagen type II mAbs (1 mg each) on d 0, followed by i.p. injection of 50 μ g LPS on d 3. Over the course of the next 3-4 d, the mice develop swollen wrists, ankles and digits. Therapeutic or control antagonist is administered i.p. 4 h prior to injection of the anti-collagen mAbs on d 0, and again 4 h prior to LPS
30 administration on d 3, and then continuing every 3rd day for the length of the experiment. Beginning on d 3, mice are evaluated for the development of arthritis. Severity of arthritis in each limb is scored using a four point system. 0=normal; 1=mild redness, slight swelling of ankle or wrist; 2=moderate swelling of ankle or wrist; 3=severe swelling including some digits, ankle, and foot; 4=maximally inflamed.

Dose Ranges

The magnitude of prophylactic or therapeutic dose of a compound of Formula I will, of course, vary with the nature of the severity of the condition to be treated and with the particular compound of Formula I and its route of administration. It will also vary according to the age, weight and response of the individual patient. In general, the daily dose range lie within the range of from about 0.001 mg to about 100 mg per kg body weight of a mammal, preferably 0.01 mg to about 50 mg per kg, and most preferably 0.1 to 10 mg per kg, in single or divided doses. On the other hand, it may be necessary to use dosages outside these limits in some cases.

For use where a composition for intravenous administration is employed, a suitable dosage range is from about 0.001 mg to about 25 mg (preferably from 0.01 mg to about 1 mg) of a compound of Formula I per kg of body weight per day and for cytoprotective use from about 0.1 mg to about 100 mg (preferably from about 1 mg to about 100 mg and more preferably from about 1 mg to about 10 mg) of a compound of Formula I per kg of body weight per day. In the case where an oral composition is employed, a suitable dosage range is, e.g. from about 0.01 mg to about 100 mg of a compound of Formula I per kg of body weight per day, preferably from about 0.1 mg to about 10 mg per kg and for cytoprotective use from about 0.1 mg to about 100 mg (preferably from about 1 mg to about 100 mg and more preferably from about 10 mg to about 100 mg) of a compound of Formula I per kg of body weight per day.

Pharmaceutical Compositions

Another aspect of the present invention provides pharmaceutical compositions which comprises a compound of Formula I and a pharmaceutically acceptable carrier.

The term "composition", as in pharmaceutical composition, is intended to encompass a product comprising the active ingredients, and the inert ingredients (pharmaceutically acceptable excipients) that make up the carrier, as well as any product which results, directly or indirectly, from combination, complexation or aggregation of any two or more of the ingredients, or from dissociation of one or more of the ingredients, or from other types of reactions or interactions of one or more of the ingredients. Accordingly, the pharmaceutical compositions of the present invention encompass any composition made by admixing a compound of Formula I, additional active ingredients, and pharmaceutically acceptable excipients.

Any suitable route of administration may be employed for providing a mammal, especially a human with an effective dosage of a compound of the present invention. For

example, oral, rectal, topical, parenteral, ocular, pulmonary, nasal, and the like may be employed. Dosage forms include tablets, troches, dispersions, suspensions, solutions, capsules, creams, ointments, aerosols, and the like.

The pharmaceutical compositions of the present invention comprise a compound of
5 Formula I as an active ingredient or a pharmaceutically acceptable salt thereof, and may also contain a pharmaceutically acceptable carrier and optionally other therapeutic ingredients. The term "pharmaceutically acceptable salts" refers to salts prepared from pharmaceutically acceptable non-toxic bases or acids including inorganic bases or acids and organic bases or acids.

10 The compositions include compositions suitable for oral, rectal, topical, parenteral (including subcutaneous, intramuscular, and intravenous), ocular (ophthalmic), pulmonary (aerosol inhalation), or nasal administration, although the most suitable route in any given case will depend on the nature and severity of the conditions being treated and on the nature of the active ingredient. They may be conveniently presented in unit dosage form and prepared by
15 any of the methods well known in the art of pharmacy. For administration by inhalation, the compounds of the present invention are conveniently delivered in the form of an aerosol spray presentation from pressurized packs or nebulisers. The compounds may also be delivered as powders which may be formulated and the powder composition may be inhaled with the aid of an insufflation powder inhaler device. The preferred delivery systems for inhalation are
20 metered dose inhalation (MDI) aerosol, which may be formulated as a suspension or solution of a compound of Formula I in suitable propellants, such as fluorocarbons or hydrocarbons and dry powder inhalation (DPI) aerosol, which may be formulated as a dry powder of a compound of Formula I with or without additional excipients.

Suitable topical formulations of a compound of formula I include transdermal devices,
25 aerosols, creams, ointments, lotions, dusting powders, and the like. In practical use, the compounds of Formula I can be combined as the active ingredient in intimate admixture with a pharmaceutical carrier according to conventional pharmaceutical compounding techniques. The carrier may take a wide variety of forms depending on the form of preparation desired for administration, e.g., oral or parenteral (including intravenous). In preparing the compositions
30 for oral dosage form, any of the usual pharmaceutical media may be employed, such as, for example, water, glycols, oils, alcohols, flavoring agents, preservatives, coloring agents and the like in the case of oral liquid preparations, such as, for example, suspensions, elixirs and solutions; or carriers such as starches, sugars, microcrystalline cellulose, diluents, granulating agents, lubricants, binders, disintegrating agents and the like in the case of oral solid

preparations such as, for example, powders, capsules and tablets, with the solid oral preparations being preferred over the liquid preparations. Because of their ease of administration, tablets and capsules represent, the most advantageous oral dosage unit form in which case solid pharmaceutical carriers are obviously employed. If desired, tablets may be coated by standard aqueous or nonaqueous techniques.

In addition to the common dosage forms set out above, the compounds of Formula I may also be administered by controlled release means and/or delivery devices such as those described in U.S. Patent Nos. 3,845,770; 3,916,899; 3,536,809; 3,598,123; 3,630,200 and 4,008,719.

Pharmaceutical compositions of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets each containing a predetermined amount of the active ingredient, as a powder or granules or as a solution or a suspension in an aqueous liquid, a non-aqueous liquid, an oil-in-water emulsion or a water-in-oil liquid emulsion. Such compositions may be prepared by any of the methods of pharmacy but all methods include the step of bringing into association the active ingredient with the carrier which constitutes one or more necessary ingredients. In general, the compositions are prepared by uniformly and intimately admixing the active ingredient with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product into the desired presentation. For example, a tablet may be prepared by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine, the active ingredient in a free-flowing form such as powder or granules, optionally mixed with a binder, lubricant, inert diluent, surface active or dispersing agent. Molded tablets may be made by molding in a suitable machine, a mixture of the powdered compound moistened with an inert liquid diluent. Desirably, each tablet contains from about 1 mg to about 500 mg of the active ingredient and each cachet or capsule contains from about 1 to about 500 mg of the active ingredient.

The following are examples of representative pharmaceutical dosage forms for the compounds of Formula 1:

	Injectable Suspension (i.m.)	mg/mL
30	Compound of Formula I	10
	Methylcellulose	5.0
	Tween 80	0.5
	Benzyl alcohol	9.0
	Benzalkonium chloride	1.0

Water for injection to a total volume of 1 mL

	Tablet	mg/tablet
	Compound of Formula I	25
	Microcrystalline Cellulose	415
5	Povidone	14.0
	Pregelatinized Starch	43.5
	Magnesium Stearate	<u>2.5</u>
		500
	Capsule	mg/capsule
10	Compound of Formula I	25
	Lactose Powder	573.5
	Magnesium Stearate	<u>1.5</u>
		600
15	Aerosol	Per canister
	Compound of Formula I	24 mg
	Lecithin, NF Liquid Concentrate	1.2 mg
	Trichlorofluoromethane, NF	4.025 g
	Dichlorodifluoromethane, NF	12.15 g
20	Combination Therapy	
	Compounds of Formula I may be used in combination with other drugs that are used in the treatment/prevention/suppression or amelioration of the diseases or conditions for which compounds of Formula I are useful. Such other drugs may be administered, by a route and in an amount commonly used therefor, contemporaneously	
25	or sequentially with a compound of Formula I. When a compound of Formula I is used contemporaneously with one or more other drugs, a pharmaceutical composition containing such other drugs in addition to the compound of Formula I is preferred. Accordingly, the pharmaceutical compositions of the present invention include those that also contain one or more other active ingredients, in addition to a compound of	
30	Formula I. Examples of other active ingredients that may be combined with a compound of Formula I, either administered separately or in the same pharmaceutical compositions, include, but are not limited to: (a) VLA-4 antagonists such as those described in US 5,510,332, W097/03094, W097/02289P W096t4O781P W096/22966, W096/20216, W096101644, W096/06108, W095/15973 and W096131206; (b) steroids	

- such as declomethasone, methylprednisolone, betamethasone, prednisone, dexamethasone, and hydrocortisone; (c) immunosuppressants such as cyclosporin, tacrolimus, rapamycin and other FK-506 type immunosuppressants; (d) antihistamines (H1-histamine antagonists) such as bromopheniramine, chlorpheniramine, dextrchlorpheniramine, triprolidine, clemastine, diphenhydramine, diphenylpyraline, tripeleminamine, hydroxyzine, methdilazine, promethazine, trimeprazine, azatadine, cyproheptadine, antazoline, pheniramine pyrilamine, astemizole, terfenadine, loratacline, cetirizine, fexofenadine, descarboethoxyloratadine, and the like; (e) non-steroidal anti-asthmatics such as P2-agonists (terbutaline, metaproterenol,

10 EXAMPLES

The following examples serve to provide further appreciation of the invention but are not meant in any way to restrict the effective scope of the invention.

Example 1: Cell Adhesion Assay Protocol

This illustrates the protocol for determining utility of the antagonists herein.

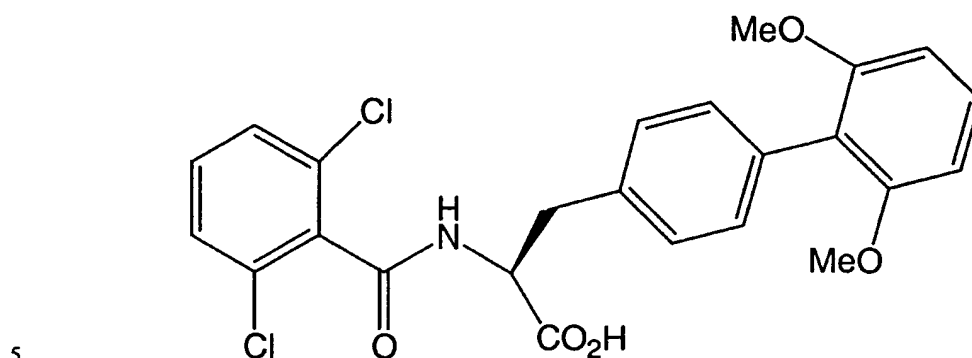
- 15 More particularly, the protocol determines the ability of such organic compounds to inhibit and prevent collagen-based cell adhesion.

Protocol:

1. Coat a 96-well plate with Collagen IV (0.5 ug/ml), Collagen I (5 ug/ml), BSA-CS1 (1 ug/ml), or Laminin (20 ug/ml) for adhesion assays, $\alpha 1\beta 1$, $\alpha 2\beta 1$, $\alpha 4\beta 1$, or $\alpha 6\beta 1$, respectively in NaBicarb. pH 9.2 at 4°C overnight.
2. Wash the plate twice with 1X PBS, 100 μ l/well.
3. Block the plate with 1% heat-treated BSA in PBS, 100 μ l/well for 1+ hr.
4. Wash the plate twice with assay buffer (TBS complete + 1 mM $MnCl_2$), 100 μ l/well.
- 25 5. Add compound (2X desired conc.) and cells (4×10^6 cells/ml, labeled with BCECF-AM [2',7'-bis(2-carboxyethyl)-5-(6)-carboxyfluorescein, acetoxymethyl ester] at 37°C for 15 min.) each at 25 μ l/well.
6. Incubate the plate at room temperature for 30 minutes.
7. Before washing the plate, read the total cells input in a fluorescent plate reader.
- 30 8. Wash the plate three times with assay buffer, 100 μ l/well.
9. Read the remaining bound cells in the fluorescent plate reader.

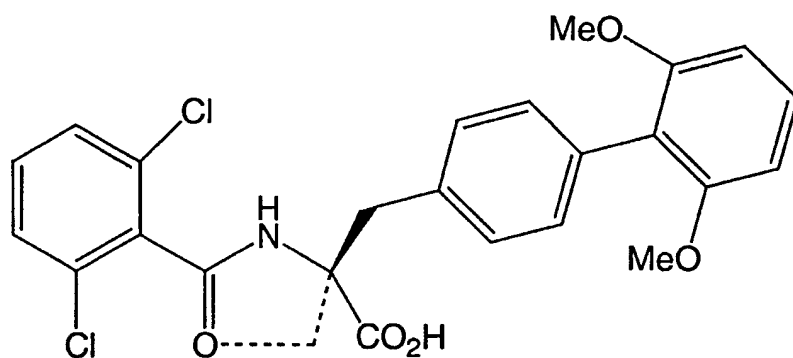
Example 2: Illustrative Synthetic Approaches

In certain embodiments, the artisan identifies the chemical structure of a compound having beta1 subunit containing integrin activity, such as, for example, the following (A):



The amide group adjacent to the acidic moiety in A is identified and phantom bonds can be formed between the carbonyl of the amide and the α carbon of the amino acid to form (B)

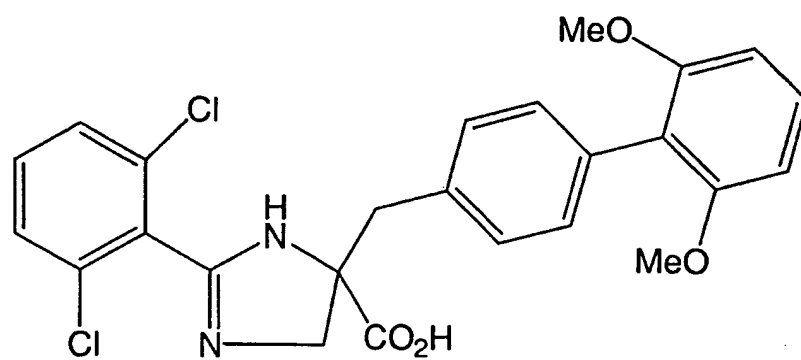
10 (B)



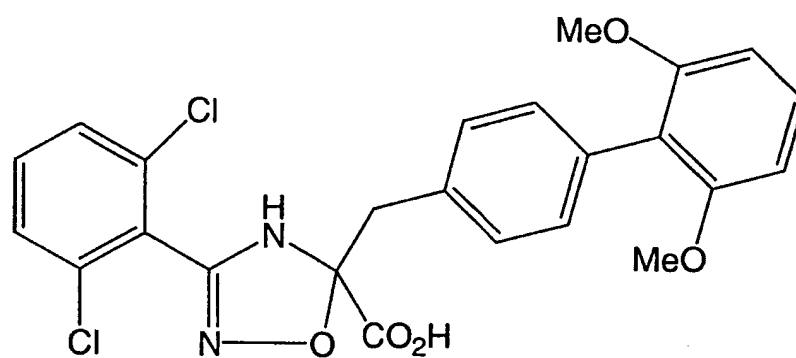
These bonds can then be converted to a heterocycle, such as (C)

15

(C)

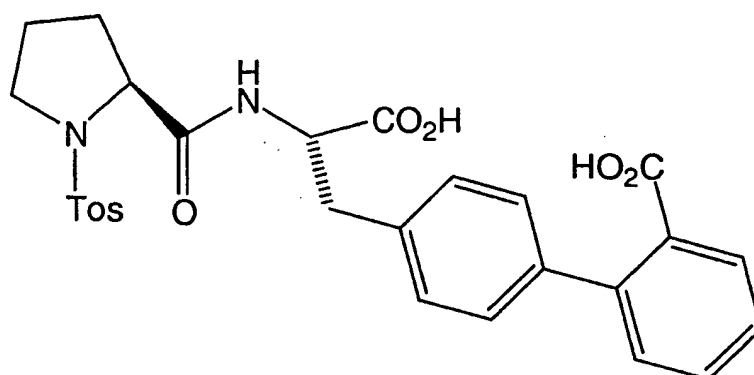


5 or (D)

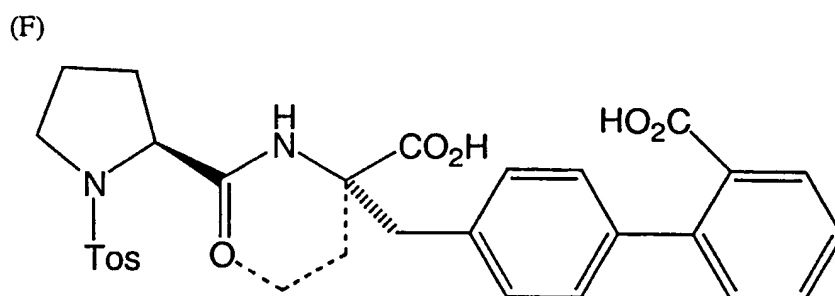


10 This procedure can also be extended to 6 membered ring heterocycles, for example (E)

(E)



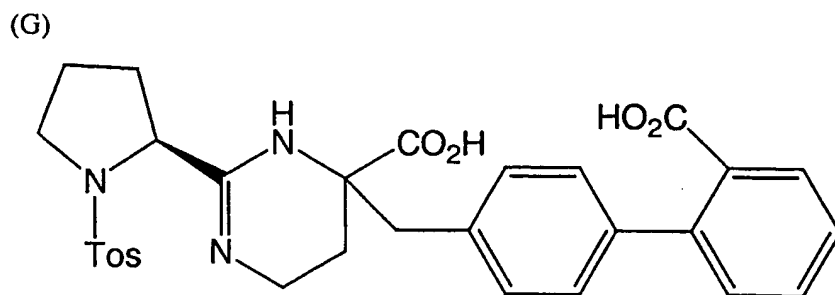
The amide group of **(E)** adjacent to the acidic moiety is identified and phantom
bonds can be formed between the carbonyl of the amide and the α carbon of the amino
5 acid **(F)**.



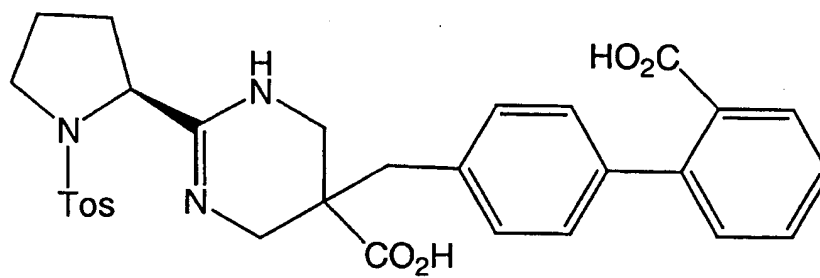
10

These bonds can then be converted to a heterocycle, such as **(G)**

15



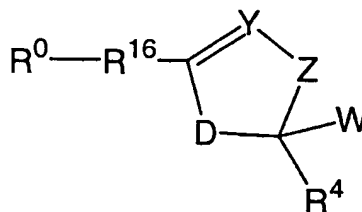
or

(H)

CLAIMS

1. An antagonist of a plurality of beta1 subunit containing integrins, the antagonist having the formula (I):

5



wherein

D is selected from

- | | | |
|----|----|---|
| 10 | 1 | $\text{NR}^j-(\text{CR}^k\text{R}^m)_m$ |
| | 2 | $\text{S}(\text{O})_n-\text{NR}^j$ |
| | 3 | NR^j-O |
| | 4 | $\text{S}-\text{S}$ |
| | 5 | NR^j-NR^j |
| 15 | 6 | $(\text{CR}^k\text{R}^m)_m-\text{O}$ |
| | 7 | $(\text{CR}^k\text{R}^m)_m-\text{NR}^j$ |
| | 8 | $\text{O}-(\text{CR}^k\text{R}^m)_m$ |
| | 9 | $(\text{CR}^k\text{R}^m)_m-(\text{CR}^k\text{R}^m)_n$ |
| | 10 | $\text{S}(\text{O})_n-(\text{CR}^k\text{R}^m)_m$ or |
| 20 | 11 | $(\text{CR}^k\text{R}^m)_m-\text{S}(\text{O})_n$ |
| | 12 | $\text{NR}^j-\text{S}(\text{O})_n$ |
| | 13 | $\text{O}-\text{NR}^j$ |
| | 14 | NR^j |
| | 15 | O |
| 25 | 16 | $\text{S}(\text{O})_n$ |
| | 17 | $\text{C}(\text{O})$ |
| | 18 | $\text{NR}^j\text{C}(\text{O});$ or |
| | 19 | $\text{C}(\text{O})\text{NR}^j$ |

30 n is an integer from 0 to 2;

m is an integer from 1 to 2;

W is selected from

- 1 $-(\text{CR}^f\text{R}^g)_n\text{C}(\text{O})\text{OR}^d$
- 2 $-(\text{CR}^f\text{R}^g)_n5\text{-tetrazolyl}$
- 5 3 $-(\text{CR}^f\text{R}^g)_n\text{S}(\text{O})_2\text{NHR}^d$

X is selected from $\text{S}(\text{O})_2$, $\text{S}(\text{O})_2\text{NR}^e$, $\text{C}(\text{O})$, $\text{C}(\text{O})\text{O}$, $\text{C}(\text{O})\text{NR}^e$, CR^fR^g

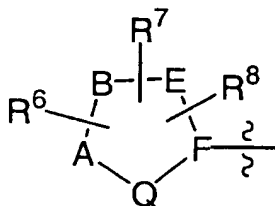
Y is selected from N, CR^i

Z is selected from

- 1 $(\text{CR}^k\text{R}^m)_n$
- 10 2 NR^j
- 3 $\text{S}(\text{O})_n$
- 4 O; or
- 5 $\text{C}(\text{O})$

R^0 is selected from R^4 and

15



A and E are independently selected from -C- and -C-C-, -C=C-;

20 F is selected from N, CR^2

Q is selected from $-\text{CR}^j$ -, $\text{C}(\text{O})$, O, $\text{S}(\text{O})_n$, NH, NXR^1

B is selected from the group consisting of

- 1 a bond,
- 2 -C-,
- 25 3 -C-C-;
- 4 -C=C-,
- 5 a heteroatom selected from the group consisting of nitrogen, oxygen, and sulfur; or
- 6 $-\text{S}(\text{O})_m$ - .

30 R^1 is

- 1 C1-10 alkyl,
- 2 C2-10alkenyl,
- 3 C2-10 alkynyl,
- 4 Cy,
- 5 C1-10 alkyl-Cy,
- 6 C2-10 alkenyl-Cy, or
- 7 C2-C10 alkynyl-Cy,

wherein alkyl, alkenyl, and alkynyl are optionally substituted with one to four substituents independently selected from R^a ; and Cy is optionally substituted with one to four substituents independently selected from R^b ;

R^2 is

- 1 hydrogen,
- 2 C1-10 alkyl,
- 3 C2-10 alkenyl,
- 4 C2-10 alkynyl,
- 5 aryl,
- 6 C1-10 alkyl-aryl,
- 7 heteroaryl, or
- 8 C1-10 alkyl- heteroaryl,

wherein alkyl, alkenyl, and alkynyl are optionally substituted with one to four substituents independently selected from R^a ; and aryl and heteroaryl are optionally substituted with one to four substituents independently selected from R^b ;

R^3 is

1. hydrogen
2. C1-10 alkyl,
3. C2-10 alkenyl,
4. C2-10 alkynyl,
5. Cy,
6. C1-10 alkyl-Cy,
7. C2-10 alkenyl-Cy or
8. C2-10 alkynyl-Cy

wherein alkyl, alkenyl and alkynyl are optionally substituted with one to four substituents selected from phenyl and R^x ; and phenyl and Cy are optionally substituted with one to four substituents independently selected from R^y

R^4 is

- 5 1. hydrogen
2. C1-10 alkyl,
3. C2-10 alkenyl,
4. C2-10 alkynyl,
5. Cy,
- 10 6. C1-10 alkyl-Cy,
7. C2-10 alkenyl-Cy or
8. C2-10 alkynyl-Cy

wherein alkyl, alkenyl and alkynyl are optionally substituted with one to four substituents selected from phenyl and R^x ; and phenyl and Cy are optionally substituted with one to four substituents independently selected from R^y or R^4 ,
15 with either R^f or R^g forming a mono- or bi-cyclic ring containing 0-2 heteroatoms selected from nitrogen, oxygen or sulfur, wherein nitrogen is optionally substituted with R^j , $C(O)R^e$, SO_2R^e or $SO_2NR^dR^e$

R^6 , R^7 , and R^8 are each independently selected from the group consisting of R^d and R^x
20 or two of R^6 , R^7 , and R^8 and the atom to which both are attached, or two of R^6 , R^7 , and R^8 and the two adjacent atoms to which they are attached, together form a 5-7 membered saturated or unsaturated monocyclic ring containing zero to three heteroatoms selected from N, O or S,

R^{12} is

- 25 1. hydrogen
2. C1-10 alkyl,
3. C2-10 alkenyl,
4. C2-10 alkynyl,
5. Cy,
- 30 6. C1-10 alkyl-Cy,
7. C2-10 alkenyl-Cy or
8. C2-10 alkynyl-Cy

Wherein alkyl, alkenyl and alkynyl are optionally substituted with one to four substituents selected from phenyl and R^x ; and Cy is optionally substituted with one to four substituents independently selected from R^y

R^{13} is

- 5 1 hydrogen,
- 2 C1-10 alkyl,
- 3 C2-10 alkenyl,
- 4 C2-10 alkynyl,
- 5 aryl,
- 10 6 C1-10 alkyl-aryl,
- 7 heteroaryl, or
- 8 C1-10 alkyl-heteroaryl,

wherein alkyl, alkenyl and alkynyl are optionally substituted with one to four substituents selected from R^x and aryl and heteroaryl are optionally substituted with one to four substituents independently selected from R^y ; or

15 R^{12} , R^{13} and the carbon to which they are attached form a 3-7 membered mono- or bicyclic ring containing 0-2 heteroatoms selected from N, O and S; wherein nitrogen is optionally substituted with R^j , $C(O)R^e$, SO_2R^e or $SO_2NR^dR^e$

R^{16} is selected from a bond, NR^j , O and $S(O)_n$

20 R^a is

- 1 Cy, or
- 2 a group selected from R^x ;

wherein Cy is optionally substituted with one to four substituents independently selected from R^c ;

25 R^b is

- 1 a group selected from R^a ,
- 2 C1-10 alkyl,
- 3 C2-10 alkenyl,
- 4 C2-10 alkynyl, or

30 5 C1-10 alkyl-aryl,

wherein alkyl, alkenyl, alkynyl, aryl, heteroaryl are optionally substituted with a group independently selected from R^c ;

R^c is

- 1 halogen,

- 2 NO₂,
 3 C(O)OR^f
 4 Cl-4 alkyl,
 5 Cl-4 alkoxy,
 6 aryl,
 7 aryl Cl-4 alkyl,
 8 aryloxy,
 9 heteroaryl,
 10 NR^fR^g,
 11 NR^fC(O)R^g,
 12 NR^fC(O)NR^fR^g,
 16 CN,
 17 C(O)Cy;
 18 C(O)alkyl;
 15 16 C(O)NR^fR^g
 17 alkyloxy; or
 18 NR^fC(O)OR^e

wherein aryl, heteroaryl and Cy are optionally substituted with 1 to 4 substituents independently selected from R^x

- 20 R^d and R^e are independently selected from hydrogen, Cl-10 alkyl, C2-10 alkenyl, C2-10 alkynyl, Cy and Cy Cl-10 alkyl, aryl, heteroaryl, aryl-substituted aryl, aryl substituted heteroaryl, heteroaryl-substituted heteroaryl

wherein alkyl, alkenyl, alkynyl, heteroaryl and Cy is optionally substituted with one to four substituents independently selected from R^c; or

- 25 R^d and R^e together with the atoms to which they are attached form a heterocyclic ring of 5 to 7 members containing 0-2 additional heteroatoms independently selected from oxygen, sulfur and nitrogen; wherein nitrogen is optionally substituted with R^j, C(O)R^c, SO₂R^c or SO₂NR^dR^e

- R^f and R^g are independently selected from R³ or R^f and R^g together with the carbon to which they are attached form a ring of 5 to 7 members containing 0-2 heteroatoms independently selected from oxygen, sulfur and nitrogen wherein nitrogen is optionally substituted with R^j, C(O)R^c, SO₂R^c or SO₂NR^dR^e
- 30

Rⁱ is

- 1 Cl-10 alkyl,

- 2 C2-10 alkenyl,
 3 C2-10 alkynyl, or
 4 aryl;

wherein alkyl, alkenyl, alkynyl and aryl are each optionally substituted with one
 5 to four substituents independently selected from R^c ;

R^j is selected from hydrogen, C1-10 alkyl, C2-10 alkenyl, C2-10 alkynyl, Cy and Cy
 C1-10 alkyl, aryl, heteroaryl, aryl-substituted aryl, aryl substituted heteroaryl,
 heteroaryl-substituted heteroaryl

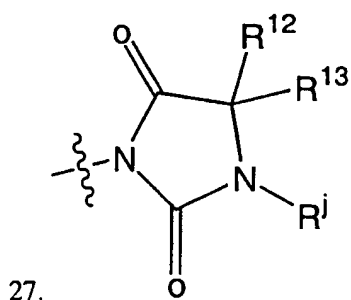
wherein alkyl, alkenyl, alkynyl, heteroaryl, and Cy is optionally substituted with one to
 10 four substituents independently selected from R^c

R^k and R^m are independently selected from hydrogen, C1-10 alkyl, C2-10 alkenyl, C2-
 10 alkynyl, Cy and Cy C1-10 alkyl, aryl, and heteroaryl

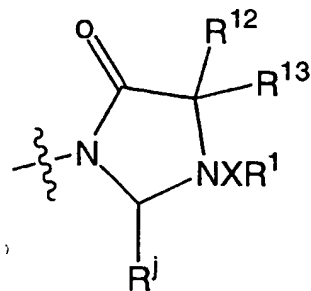
R^x is

- 1 -O R^d ,
 15 2 -NO₂,
 3 halogen,
 4 -S(O)_m R^d
 5 -S R^d ,
 6 -S(O)₂OR^d,
 20 7 -S(O)_m N R^d R^e ,
 8 -N R^d R^e ,
 9 -O(C R^f R^g)_n N R^d R^e ,
 10 -C(O) R^d ,
 11 -CO₂ R^d ,
 25 12 -CO₂(C R^f R^g)_n CON R^d R^e ,
 13 -OC(O) R^d ,
 14 -CN,
 15 -C(O)N R^d R^e ,
 16 -N R^d C(O) R^e ,
 30 17 -OC(O)N R^d R^e ,
 18 -N R^d C(O)O R^e ,
 19 -N R^d C(O)N R^d R^e ,
 20 -C R^d (N-O R^e),
 21 -CF₃,

- 22 oxo,
 23 $\text{N R}^d \text{C(O)N R}^d \text{SO}_2 \text{R}^i$,
 24 $\text{N R}^d \text{S(O)}_m \text{R}^e$,
 25 $-\text{OS(O)}_2 \text{O R}^d$,
 5 26 $-\text{OP(O)(O R}^d)_2$;



- 28 $-\text{N R}^d \text{C(S)N R}^d \text{R}^e$, or



R^y is

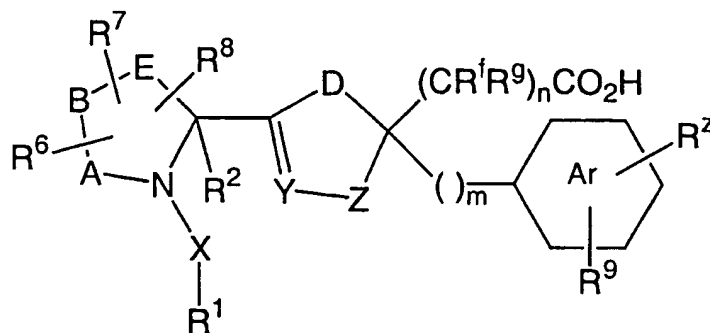
- 1 a group selected from R^x ,
 2 C1-10 alkyl,
 15 3 C2-10 alkenyl,
 4 C2-10 alkynyl,
 5 aryl C1-10 alkyl- aryl,
 6 C1-10 alkyl- heteroaryl,
 7 cycloalkyl,
 20 8 heterocyclyl
 9 aryl, or
 10 10 heteroaryl

wherein alkyl, alkenyl, alkynyl, heteroaryl and aryl are each optionally substituted with one to four substituents independently selected from R^x ; and

Cy is cycloalkyl, heterocycyl, aryl, or heteroaryl;

2. An antagonist of a plurality of integrins, the antagonist having the formula (II):

5



where Ar is aryl, heteroaryl, aryl-substituted aryl, aryl substituted heteroaryl which are substituted with R^z and R^9 .

10 R^9 is selected from H and R^y

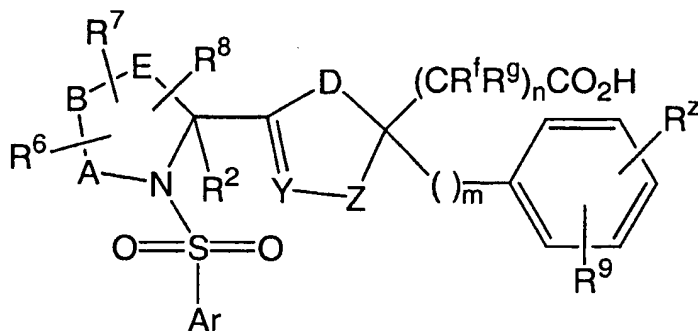
R^z is selected from

- 1 OR^d
- 2 NHR^d
- 3 $NR^dS(O)_mR^c$
- 4 $NR^dC(O)R^c$

15

and all other designations and substituents are as previously recited in claim 1.

3. An antagonist of a plurality of integrins, the antagonist having the formula (III):



20

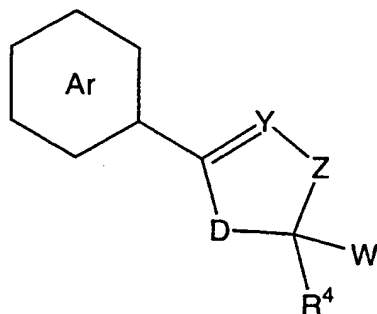
wherein Ar is aryl, heteroaryl, aryl-substituted aryl, aryl substituted heteroaryl which are optionally substituted with one to four substituents independently selected from R^x and R^9 is selected from H and R^y .

R^z is selected from

- 5 1 OR^d
- 2 NHR^d
- 3 $NR^dS(O)_mR^e$
- 4 $NR^dC(O)R^e$

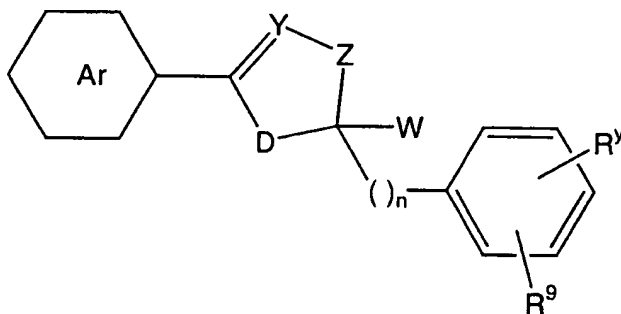
and all other designations and substituents are as previously recited in claim 1.

- 10 4. An antagonist of a plurality of integrins, the antagonist having the formula (IV):



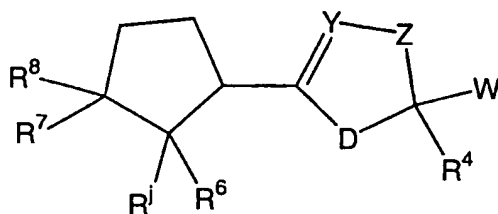
- 15 wherein Ar is selected from aryl, heteroaryl, aryl substituted aryl, aryl substituted heteroaryl, and heteroaryl substituted heteroaryl and further wherein said aryl, heteroaryl, aryl substituted aryl, aryl substituted heteroaryl, heteroaryl substituted heteroaryl are optionally substituted with 1 to 4 substituents independently selected from R^x and all other designations and substituents are as recited in claim 1.

5. An antagonist of a plurality of integrins, the antagonist having the formula (V):



wherein Ar is selected from aryl, heteroaryl, aryl substituted aryl, aryl substituted heteroaryl, and heteroaryl substituted heteroaryl and further wherein said aryl, heteroaryl, aryl substituted aryl, aryl substituted heteroaryl, heteroaryl substituted heteroaryl are optionally substituted with 1 to 4 substituents independently selected from R^x , R^y is selected from H and R^y and all other designations and substituents are as recited in claim 1

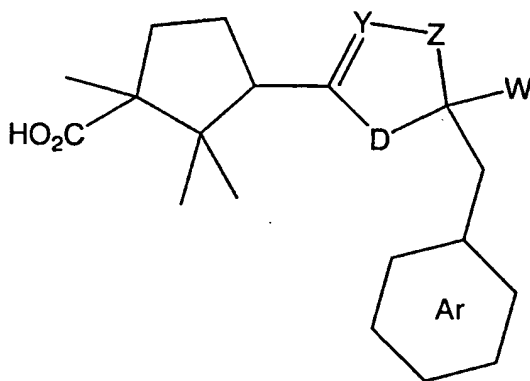
6. An antagonist of a plurality of integrins, the antagonist having the formula (VI):



15

wherein all designations and substituents are as recited in claim 1.

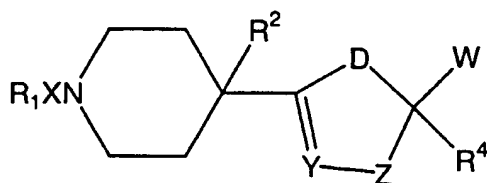
7. An antagonist of a plurality of integrins, the antagonist having the formula (VII):



20 wherein Ar is selected from aryl, heteroaryl, aryl substituted aryl, aryl substituted heteroaryl, and heteroaryl substituted heteroaryl and further wherein said aryl, heteroaryl, aryl substituted aryl, aryl substituted heteroaryl, heteroaryl substituted heteroaryl are optionally substituted with 1 to 4 substituents independently selected from R^x , R^y is selected from H and R^y and all other designations and substituents are as recited in claim 1

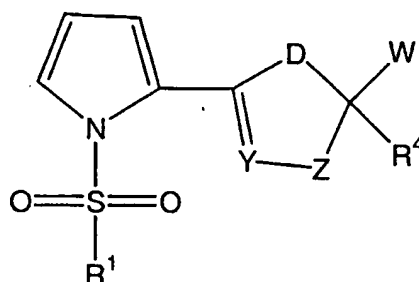
heteroaryl are optionally substituted with 1 to 4 substituents independently selected from R^x and all other designations and substituents are as recited in claim 1.

8. An antagonist of a plurality of integrins, the antagonist having the formula (VIII):



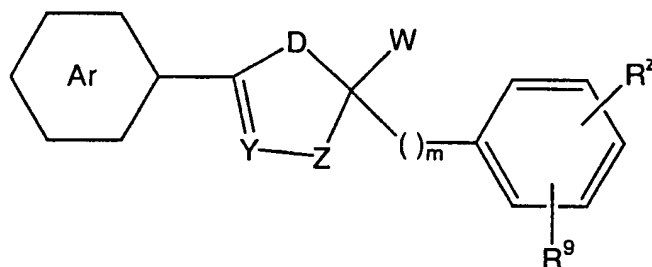
wherein all designations and substituents are as recited in claim 1.

9. An antagonist of a plurality of integrins, the antagonist having the formula (IX):



wherein all designations and substituents are as recited in claim 1.

10. An antagonist of a plurality of integrins, the antagonist having the formula (X):



Ar is selected from aryl, heteroaryl, aryl substituted aryl, aryl substituted heteroaryl, and heteroaryl substituted heteroaryl and further wherein said aryl, heteroaryl, aryl substituted aryl, aryl substituted heteroaryl, heteroaryl substituted heteroaryl are optionally substituted with 1 to 4 substituents independently selected

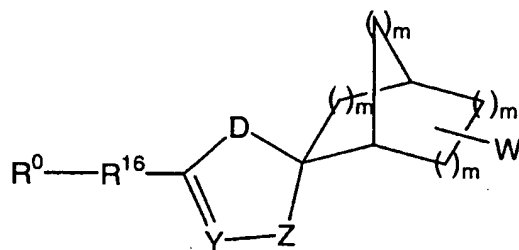
from R^x , R^9 is selected from H and R^y ,

R^Z is selected from

- 1 OR^d
- 2 NHR^d
- 3 $\text{NR}^d\text{S(O)}_m\text{R}^e$
- 4 $\text{NR}^d\text{C(O)R}^e$

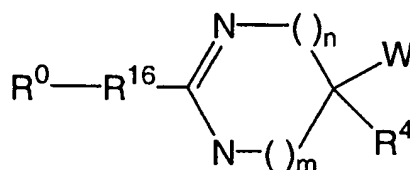
5 and all other designations and substituents are as recited in claim 1.

11. An antagonist of a plurality of integrins, the antagonist having the formula (XI):



10 wherein all designations and substituents are as recited in claim 1.

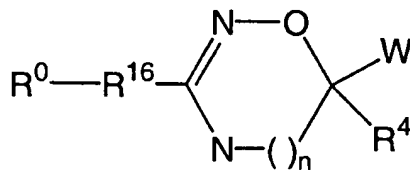
12. An antagonist of a plurality of integrins, the antagonist having the formula (XII):



15

wherein all designations and substituents are as recited in claim 1.

13. An antagonist of a plurality of integrins, the antagonist having the formula (XIII):



20

wherein all designations and substituents are as recited in claim 1.

14. The antagonist of claims 1-13, wherein said plurality of integrins is a plurality of beta 1-subunit containing integrins.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 01/03347

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07D233/28 C07D271/06 C07D403/04 A61K31/415 A61K31/41
A61K31/505 A61P43/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, BEILSTEIN Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 00 00477 A (MILICI ANTHONY JOHN ;PFIZER PROD INC (US); CHUPAK LOUIS STANLEY (U) 6 January 2000 (2000-01-06) claim 1 -----	1

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

G document member of the same patent family

Date of the actual completion of the international search

14 May 2001

Date of mailing of the international search report

25.05.01

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Gettins, M

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 01/03347

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☒ Claims Nos.: 6-9, 11
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this International application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 6-9,11

The attempt to limit the scope of the compound claims by reference to the ability to antagonise betal subunit containing integrins cannot be seen a helpful limitation in compound claims. The compounds which actually have this property can only be determined by an exhaustive process of trial and error which places an undue burden upon the skilled person trying to determine the precise scope of the claims. as a result of this definition the claims also lack clarity (Article 6 PCT). An attempt is made to define the compounds by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible.

Present claims 1-14 relate to an extremely large number of possible compounds. In fact, the claims contain so many options, variables, possibilities, independent compound claims and permutations that a lack of clarity (and/or conciseness) within the meaning of Article 6 PCT arises to such an extent as to render a meaningful search of the claims impossible. Given the way the application has been drafted it is impossible to be certain whether or not the application is actually meant to contain any examples. For the purposes of the search it is assumed that (C) and (D) on page 41 are examples of formula (I) in claim 1. Page 41 refers to (E) being 6 membered heterocycles, but this is contradicted by the formula on page 42 so it is not clear what the structure of (E) is meant to be. (G) on page 42 and (H) on page 43 appear to be examples designed to fall within the scope of claims 1-5.

Additionally support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the compounds. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. The only ever present feature is the ring containing Y, Z and D. Since the scope of this ever present feature is huge and cannot be considered to be a representative generalisation of the examples the search has been carried out for those parts of the claims which appear to be supported and disclosed, namely those parts relating to the examples (C), (D), (G) and (H). No other compounds or the activity thereof have been searched.

It is additionally pointed out that a full assessment of unity cannot be made given the limited scope of the search.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 01/03347

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0000477 A	06-01-2000	AU 3841699 A	17-01-2000
		BR 9911701 A	20-03-2001
		EP 1091943 A	18-04-2001
		NO 20006600 A	21-02-2001